DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00012-31 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 charecters or less. Title must fit on one line between the borders.)

Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

B.O. Fowler, Research Chemist, BRB, NIDR

COOPERATING UNITS (if any)

ADAHF, NIST, Gaithersburg, MD; NIST, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (hydroxide, fluoride, chloride, carbonate, water and acid phosphate). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependence and polarization) are then utilized to establish compositional and structural details of the apatites in question, which include: the type and geometry of constituent ions; the site or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00074-21 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone and Tooth Matrix Biochemistry and Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

L.W. Fisher, Research Chemist, BRB, NIDR
D.I. Deutsch, Visiting Scientist, BRB, NIDR
J.T. Stubbs, IRTA Fellow, BRB, NIDR
H. Green, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

Sackler School of Medicine, Tel Aviv, Israel; University of New Mexico, Albuquerque, NM; Universita "La Sapieza", Rome, Italy; Dental Research Unit, Hebrew University, Jerusalem, Israel

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.76	2.59	.17

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Protein Biochemistry Group was involved in six major projects in FY 1992. Two projects, 1) the human decorin gene cloning, sequencing and chromosomal localization and 2) preliminary production and characterization of functional fragments of the integrin-binding bone sialoprotein (BSP) were brought to successful interim conclusions (Vetter et al., 1993 and Mintz et al., 1993 respectively). The discovery that human decorin has two exons-1 and therefore two independent promoters is continuing to be an area that we are exploring (Fisher, 1993). With our collaborators, the closely related biglycan gene has been eliminated as the genetic defect in the human disease Happle Syndrome (a chondrodysplasia punctata with streaky hyperkeratosis) (Traupe et al., 1993), although the gene maps very close to this disorder on the X chromosome.

Our attempts to clone the dentin phosphophoryn, project 3, was as unsuccessful as that of several other laboratories around the world. Due to the importance of this molecule to the dental community, however, we continue to try new approaches. A new project was started (number 4) upon the arrival of Dr. John Stubbs from the graduate school of Rutgers University. This project involves the uncovering of the functions of the propeptides of the two small proteoglycans, decorin and biglycan. Project 5 is the cloning, sequencing and chromosomal localization of a cytoskeletal protein, drebrin. This gene product was originally thought to be a neurite-specific protein involved in dendrite formation. We think that this product may be involved in the formation of similar cellular extensions in the osteocyte, the arborizing lacunae. With Dr. Dan Deutsch, a visiting scientist from Hebrew University, Israel, we have conducted the final major project for 1992, the cloning, sequencing and chromosomal localization of the human enamel gene, tuftelin. At this time the gene is fully cloned and partially sequenced. The gene has been localized to chromosome 1, an interesting finding given that most of the cases of amelogenesis imperfecta (AI) are autosomal in inheritance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00088-20 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical, Structural and Morphological Studies on Calcium Phosphates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

E.D. Eanes, Chief, MCSS, BRB, NIDR
A.W. Hailer, Chemist, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	.4	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study the physical, chemical, and ultrastructural properties of calcium phosphate salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable calcium phosphate salts to carry out their specialized role *in vivo*. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. The principal endeavor currently being pursued involves artificial lipid vesicles (liposomes) as *in vitro* models to investigate physico-chemical aspects of matrix vesicle (MV)-mediated calcification *in vivo*. The latest phase of this endeavor, a study that examined the modulating effect of cartilage matrix proteoglycans (aggrecan) on mineral development in the liposomal model system, was completed during the report period. Findings from this study showed that enzymatic breakdown of extraliposomal proteoglycans (PG) does not necessarily destroy the retarding effect PGs have on calcium phosphate precipitation in liposomal suspensions. Core protein as well as glycosaminoglycan components, but not hyaluronic acid, are equally as effective as intact PG in delaying precipitate development. On the other hand, the breakdown products of chondroitinase digestion of the glycosaminoglycan components and of proteinase digestion of the core protein do not have a strong inhibitory effect on the precipitation. These data suggest that the core protein and glycosaminoglycan chains may have to be destroyed before PG, when freely suspended in solution, loses its inhibitory influence on biomineral development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00157-18 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Studies of the Structure and Dynamics of Staphylococcal Nuclease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

D.A. Torchia, Chief, PBS, BRB, NIDR
T. Yamazaki, Staff Fellow, BRB, NIDR
L.K. Nicholson, IRTA Fellow, BRB, NIDR

COOPERATING UNITS (if any)

University of Maryland; LCP, NIDDK; CUNY

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.95	1.95	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NMR studies of the structure and dynamics of staphylococcal nuclease (SNase) were carried out in order to elucidate the catalytic function of this model enzyme. We studied (1) the molecular dynamics of wild type SNase and (2) the three dimensional (3D) structure of SNase mutants.

(1) SNase dynamics. Heteronuclear 2D NMR pulse sequences were used to obtain accurate values of ^{15}N and ^{13}C labeled sites in SNase. The relaxation parameters of the 14 alanine, 22 leucine, and 4 methionine methyl carbons in SNase were measured and analyzed using the model-free approach. Little internal motion was found at the α and β sites of the alanine residues. In contrast significant internal motion of nearly one half of the leucine sidechains was observed in SNase liganded to Ca^{2+} and pdTp. In the absence of the ligands, internal motions increased substantially. These results were interesting because all leucine sidechains are buried, suggesting a dynamic environment in portions of the protein interior. In addition, two of the methionine residues near the protein surface, showed substantially greater internal mobility than the most flexible leucine sidechains.

(2) Mutant structure and function. The SNase mutant, G50F/V51N ΔSNase, is considerably more stable than the wild type enzyme, but is ca. 100 fold less active. We have determined the 3D structure in solution to high resolution based on over 2000 NOE constraints. The structures of the mutant and wild type proteins are essentially identical except for a few residues near the active site. The difference in stability is due to the replacement of a disordered loop of the wild type enzyme with a well structured tight turn in the mutant. The lower activity of the mutant protein appears to result from the movement of the active site Glu-43 sidechain from its position in the wild type structure.

The significance of the project lies in the information about (1) the range and time scales of protein structural fluctuations provided by dynamics studies and (2) the relationship between structure and function that comes from comparing the structures of wild type and mutant proteins. In addition, many novel NMR experiments have been developed using SNase as a model system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00379-10 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Bone Matrix Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

M.F. Young, Research Biologist, BRB, NIDR
J.M. Kerr, Staff Fellow, BRB, NIDR
K. Ibaraki, Visiting Associate, BRB, NIDR
A.M. Heegaard, Visiting Fellow, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The matrix proteins of bones and teeth play key roles in the structure and function of these tissues. Our objective is to study the structure and function of these macromolecules and to understand the regulation of their expression. The structures of bone and tooth matrix proteins have been studied by constructing recombinant cDNA libraries from bone or ameloblast cell mRNA. cDNAs encoding several bone and tooth matrix proteins were isolated. The clones and antibodies were used to determine the primary structure and mode of expression of the genes in cultured cells and intact tissue. The corresponding genomic DNAs have also been isolated and used to determine the intron-exon organization of these genes and the elements that potentially regulate their expression during development. Studies are underway using transgenic mice to identify the function of the matrix proteins and the elements that regulate their expression during development and aging *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00380-10 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Bone Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P. Gehron Robey, Biologist, BRB, NIDR
T.E. Hefferan, Biol. Lab Technician, BRB, NIDR
W.J. Grzesik, Visiting Associate, BRB, NIDR
A.J. Friedenstein, Visiting Scientist, BRB, NIDR
G. van der Pluijm, Visiting Fellow, BRB, NIDR
C. Crescioli, Visiting Fellow, BRB, NIDR
S. Kuznetsov, Visiting Associate, BRB, NIDR
M. Atkinson, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

Department of Biopathology, Universita "La Sapienza", Rome, Italy

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.40	4.74	0.66

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary goals are to determine the composition and functional features of the supramolecular complex of proteins that calcifies, and how cells regulate this process. Towards these aims, cell cultures that form mineralized tissues were established for biochemical analysis, and for studies at the genomic level in collaboration with Drs. Marian F. Young and Larry W. Fisher. Intrinsic factors were found to influence the biosynthetic output of these cells such as the animal species, position within the cell cycle, and importantly, the developmental age of the donor. Bone cells and their products were compared to those from other tissues, and from patients with different diseases. By histochemistry at the EM level, BSP was found in packets of matrix that, upon secretion, were the first sites of mineral deposition. Many of the bone matrix proteins, including BSP, were found to support cell attachment in vitro. By immunohistochemistry, the RGD-containing proteins and their receptors were found to be expressed at particular stages of maturation in vivo. Interestingly, one of the cell surface recept subunits that binds to fibronectin (alpha-4) was highly expressed in the osteoblastic layer. In cells from osteogenesis imperfecta patients, there were changes in post-translational modifications of not only collagen, but also proteoglycans, and in the absolute and relative amounts of the various components. These changes may cause the altered crystal structure found in OI bone, which ultimately results in fragility. In Turner's syndrome (karyotype 45, XO and characterized by short stature and early onset osteoporosis), biglycan (whose gene is on the X chromosome) was found to be reduced by 50%. In various forms of osteosarcoma in rats and humans, both qualitative and quantitative differences in bone matrix proteins were detected, which may contribute to derangements in growth and ultrastructure observed in these tumors. Continued characterization of the interrelationship between cells and their extracellular environment will provide a clearer understanding of bone metabolism in health and in disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00507-04 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structural Studies of TGF-β1 and HIV-1 Protease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

D.A. Torchia, Chief, PBS, BRB, NIDR
S. Archer, Guest Researcher, BRB, NIDR
L.K. Nicholson, IRTA Fellow, BRB, NIDR

COOPERATING UNITS (if any)

LCP, NCI; Celtrix Laboratories; R&D Systems

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.95	.95	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two HIV related proteins, TGF-β1 and the HIV-1 protease, were studied by NMR spectroscopy.

(1) TGF-β1 structure. TGF-β1 up and down regulates proliferation of HIV infected cells. In order to understand the activity of this multifunctional cytokine, we have undertaken to determine the solution structure of TGF-β1 in solution. Previously we had determined the secondary structure of the protein in solution, and shown that the solution structure is in agreement with an independently determined X-ray structure of TGF-β2, except in a domain that distinguishes the activities of the β1 and β2 isoforms. A low resolution folded structure of TGF-β1 has been determined, and we are using the X-ray structure in further interpret our NOE data. We plan to make a detailed comparison of the three dimensional solution and crystal structures in order to derive a basis for understanding differences in function of the two protein isoforms.

(2) HIV-1 protease. Several months ago we completed a material transfer agreement with the DuPont Merck Pharmaceutical Co. in which DuPont Merck agreed to provide Dr. Paul Wingfield of the NIH Protein Expression Lab with E. coli transformed to express high levels of HIV1 protease, as well as strong inhibitors of the protein. Dr. Wingfield has just supplied us with the first samples of the inhibited protease, and we have obtained high quality NMR spectra that show the protein to be properly folded an stable in solution. With a stable supply of high grade material available for the first time we are beginning 3- and 4-dimensional NMR experiments that will enable us to determine the 3D structure of the inhibited protease.

The significance of this project arises from the unique, detailed structural information that is being obtained about HIV related proteins in solution. This information will form the basis for a rational drug design based upon the understanding of the function of these proteins in terms of interactions at the molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00510-04 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Cartilage Matrix Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

T.I. Morales, Expert, BRB, NIDR
P.E. Long, Biologist, BRB, NIDR
M. Montgomery, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

Laboratory of Chemoprevention, NCI, NIH

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.28	1.0	1.28

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long term objective of this program is to elucidate the intrinsic regulatory mechanisms that control the structure and function of the resilient layer of articular cartilage that covers and protects the ends of bone. We showed that TGF-β has the ability to prevent the spontaneous proteoglycan loss that occurs in basal cartilage organ cultures by increasing biosynthetic rates and decreasing degradation. We proposed that TGF-β is an intrinsic modulator of cartilage proteoglycan metabolism and obtained a line of evidence to support this hypothesis. For example, we showed that TGF-β is a strong antagonist of the most powerful known resorptive agent for cartilage, retinoic acid. This vitamin increases catabolism and depresses rates of re-deposition. In the original experiments, the two signaling factors were added together to the cartilage explants. We have now modified our protocols to ask whether TGF-β is an effective repair agent following retinoic acid treatment. After a week of treatment with retinoic acid, proteoglycan synthesis falls to ~10% of controls and recovery following removal of the retinoid from the medium is inadequate (an average 2-fold increase over retinoid treated samples). Recovery is greatly improved by TGF-β (10-20 fold increase). However, the rates of synthesis in the presence of TGF-β following retinoid exposure never reach the levels of control cultures treated with the cytokine. The effect of IGF-1 mirrors that of TGF-β. However, when both TGF-β and IGF-1 are added to the recovering cultures, the anabolic rates rise to levels comparable to those of similarly treated control cultures. Our studies have uncovered an additive response between TGF-β and IGF-1 and indicated that this interaction should be taken into account for the future design of *in vivo* repair treatments following matrix injury, such as occurs during osteoarthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00546-02 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hyaluronic Acid Synthesis by Ovarian Cumulus and Granulosa Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

V.C. Hascall, Chief, PCS, BRB, NIDR
M. Yanagishita, Visiting Scientist, BRB, NIDR
A. Camaioni, Visiting Fellow, BRB, NIDR

COOPERATING UNITS (if any)

2nd University of Rome, Rome, Italy

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study the biological and biochemical processes involved in synthesizing and organizing the hyaluronic acid-rich extracellular matrix which surrounds most mammalian oocytes at the time of ovulation. This matrix is produced primarily by the ~1,000 cumulus cells which are initially closely adherent to the oocyte. In response to a gonadotropin surge these cells initiate hyaluronic acid synthesis and deposit it in the extracellular matrix. This process enlarges the cumulus cell-oocyte complex, and the fully expanded complex is ovulated ~10 hours later. We are studying this process with mouse cumulus cell-oocyte complexes in vitro. Three factors have been identified that are necessary for expansion: (1) a soluble factor produced by the oocyte which induces hyaluronic acid synthesis, (2) FSH (or cAMP) which amplifies the synthetic response, and (3) a factor in serum required to retain the newly synthesized hyaluronic acid in the matrix. We have shown that: a) the serum factor, identified by others as an inter-α-trypsin inhibitor, must be continuously present to maximize retention of newly synthesized hyaluronic acid in the matrix, b) decasaccharides but not octasaccharides of hyaluronic acid can displace the HA and prevent matrix formation, c) conversely, once formed decassacharides do not facilitate matrix disassembly, and d) an endogenously synthesized 45 kDa protein binds to the hyaluronic acid during matrix formation. Topics of present interest include: (1) identifying the factor produced by the oocyte that is required to induce hyaluronic acid synthesis, (2) determining how the cumulus cells respond to this factor via second messenger systems, (3) identifying the ~45 kDa protein by immunological and molecular biological probes, and (4) determining the role of this protein in matrix formation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00547-02 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Novel Methods for Analyzing Glycosaminoglycan Substructure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

V.C. Hascall, Chief, PCS, BRB
A. Calabro, Staff Fellow, PCS, BRB
M. Yanagishita, Visiting Scientist, PCS, BRB
C.K. Ng, Visiting Fellow, PCS, BRB

COOPERATING UNITS (if any)

University of Iowa, Iowa City, IA

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to develop new methods for analyzing the substructure of glycosaminoglycans with high resolution and high sensitivity. High performance anion exchangers with exceptional stability and resolving power have been developed by Dionex (CarboPac PA1) for separation of sugars and oligosaccharides. When used with appropriate monitors (UV, fluorescence, or pulsed amperometric detectors), detection limits in the ng range can be achieved. Glycosaminoglycans can be selectively degraded with enzymes: chondroitinases digest chondroitin sulfates and hyaluronic acid, heparinases digest heparin and heparan sulfate, and keratanases digest keratan sulfate. The major products are mixtures of mono-, di- and trisaccharides, with various positions carrying sulfate residues. We have developed a reductive method for introducing a fluorochrome on the reducing ends of the digestion products. This both stabilizes them to alkali and eliminates the α and β anomers. It also provides a highly sensitive fluorescent assay for detecting all the products, thereby avoiding the low detection limits of the other procedures for saturated (UV) or highly sulfated (pulsed amperometric) products. The method has been optimized with disaccharides generated from chondroitin sulfate and hyaluronic acid by the chondroitinases, and disaccharides generated from keratan sulfate with keratanases. Topics of present interest include: (1) adaptation of the method to resolve and purify oligosaccharides with different lengths from partial digests of hyaluronic acid with lyase and eliminase enzymes specific for this glycosaminoglycan, (2) resolution and identification of the disaccharides generated from heparan sulfate with various enzymes specific for this glycosaminoglycan, and (3) the application of the procedure to analyze the contents and compositions of the chondroitin sulfate and hyaluronic acid in synovial fluid samples from patients with osteoarthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00548-02 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Proteoglycan Biosynthesis in the Golgi Apparatus Using Brefeldin A

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

Masaki Yanagishita, Visiting Scientist, BRB, NIDR
Lars Uhlin-Hansen, Guest Researcher, BRB, NIDR
Anthony Calabro, Staff Fellow, BRB, NIDR
Vincent Hascall, Chief, PCS, BRB, NIDR

COOPERATING UNITS (if any)

University of Tromso, Norway

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.28	1.28	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The glycosaminoglycan components of proteoglycans are biosynthesized and modified in the golgi apparatus by highly organized carbohydrate transfer enzymes and sulfotransferases. The purpose of this project is to investigate the functional organization and subcellular localization of these enzyme complexes. Brefeldin A is a chemical which specifically blocks anterograde protein transport within the golgi apparatus. It was used to disrupt the normal biosynthetic processes for adding glycosaminoglycan chains onto proteoglycans. When ovarian granulosa cells were treated with Brefeldin A, dermatan sulfate proteoglycan synthesis was abolished whereas heparan sulfate proteoglycan synthesis was only partially inhibited, suggesting that dermatan sulfate and heparan sulfate assembly on proteoglycans occurs in different subcellular compartments. The finding that only normal heparan sulfate protein core proteins were substituted with heparan sulfate chains in the presence of the drug indicated that glycosylation enzymes are highly specific to core proteins. Topics of present interest include elucidation of core protein structure which determines highly specific glycosylation enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00549-02 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Cell Surface Heparan Sulfate Proteoglycans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

Masaki Yanagishita, Visiting Scientist, BRB, NIDR
Duncan Hiscock, Visiting Fellow, BRB, NIDR
Vincent Hascall, Chief, PCS, BRB, NIDR
Mariko Uoshima, Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

Department of Microbiology and Immunology, University of Michigan; Division of Cytokine Biology, CBER, FDA

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.53	1.53	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cell surface heparan sulfate proteoglycans are widely distributed throughout animal tissues, and are involved in critical cell functions such as cell-cell and cell-extracellular matrix interactions. Their interaction with a variety of molecules including growth factors, viruses, and extracellular matrix proteins, have important biological functions. The purpose of this project is to study the metabolism of cell surface heparan sulfate proteoglycans with focus on mechanisms involved in their endocytosis and subsequent intracellular processing. Topics of present interest include: (1) characterization of the intracellular trafficking and processing pathway for the dermatan sulfate proteoglycan which may enter the nuclear compartment; (2) further development of the procedure to isolate quantitatively and purify nuclei from UMR 106 osteoblastic cells and granulosa cells; and (3) define the intracellular subcompartments where the intercalated heparan sulfate proteoglycans are selectively degraded; (4) study functional roles of cell surface heparan sulfate proteoglycans in infection processes by human immunodeficiency virus and herpes simples virus-I, and (5) study functions of cell surface heparan sulfate proteoglycans in oral epithelial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00550-02 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis and Extracellular Matrix Organization of Hyaluronic Acid

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

Masaki Yanagishita, Visiting Scientist, BRB, NIDR
Yumi Imai, Visiting Associate, BRB, NIDR
Juan Carlos Calvo, Visiting Scientist, BRB, NIDR
Vincent Hascall, Chief, PCS, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.07	2.07	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hyaluronic acid (HA) is a unique glycosaminoglycan. Unlike others, which are synthesized on core proteins in the golgi to form proteoglycans, HA is not assembled on a core protein. Rather, it is synthesized at sites associated with the plasma membrane with the elongating chain being extended into the extracellular matrix. The biological functions of HA in the extracellular matrix are based on the ability of the HA molecules to occupy large hydrodynamic domains and to interact with various specific proteins which constitute structural components in the matrix or are associated with the cell surface. There are conflicting data concerning the nature of the HA synthetase, whether it is a single enzyme or a multi-enzyme complex. Additionally, studies on the regulation of HA synthesis have been technically difficult. The purpose of this project is to study the properties of the HA synthesizing enzyme(s) and the organization and biological roles of HA in the extracellular matrix in model cell culture systems. Topics of current interest include: determination of the mechanism by which newly synthesized HA is organized into a highly viscoelastic network during differentiation of 3T3 L1 cells into adipocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00552-02 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Differentiation Factors In Cartilage and Bone Formation and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

F.P. Luyten, Visiting Scientist, BRB, NIDR
S. Vukicevic, Visiting Scientist, BRB, NIDR
P. Chen, Visiting Associate, BRB, NIDR
M. Krosin, Biological Aide, BRB, NIDR
S. Chang, Pre-IRTA, BRB, NIDR
B. Hoang, Special Volunteer, BRB, NIDR

COOPERATING UNITS (if any)

School of Medicine, Zagreb, Croatia; CBER, FDA; Johns Hopkins, Baltimore, MD

LAB/BRANCH

Bone Research Branch

SECTION

Bone Cell Biology Group, Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.266	1.60	1.666

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to study the cartilage and bone inducing factors and to define their role in embryogenesis and in postnatal life, both in tissue formation and in disease. As tissue regeneration recapitulates the developmental sequence of embryonic tissue formation, it is conceivable that understanding the mechanisms of action of the soluble differentiation factors is a key step towards biologically controlled regeneration of skeletal tissues. This will have a significant impact on the treatment of congenital and/or acquired skeletal diseases such as large bone defects, impaired fracture healing, osteoarthritis, osteoporosis and periodontitis. This project focuses on the further characterization of cartilage and bone inducing molecules and their biological activities. Protein fractions with cartilage and bone inducing activity in vivo, have been isolated from articular cartilage and purified to homogeneity; protein sequencing data from tryptic peptides have been obtained; databank searches did not reveal homology with any known sequences. The tissue specific localization of the new protein preparation was shown by immunolocalization using a polyclonal antibody against the N-terminal sequence. Further characterization by cDNA cloning is in progress. Recombinant transient expression of isolated cDNA clones is ongoing. Using the molecular probes, we are studying their respective contribution to cartilage, endochondral and membranous bone formation. Immunohistochemical localization and in situ hybridization of cartilage and bone inducing proteins, as well as studies in vitro, indicate the selective contribution of these molecules to initiation, enhancement, maintenance and maturation of the chondrocytic and osteoblastic phenotype. This work contributes to the basic understanding of de novo cartilage and bone formation, and sets the stage for the proper indication and use of these soluble differentiation factors in a clinical setting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00554-02 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physicochemical Studies on Calcium Phosphate Cements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

E.D. Eanes, Chief, MCSS, BRB, NIDR

COOPERATING UNITS (if any)

Dental and Medical Materials Group, Polymers Division, NIST, Gaithersburg, MD; ADAHF Paffenbarger Research Center, NIST, Gaithersburg, MD; Tokushima University, Japan

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.3	.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Self-setting calcium phosphate cements (CPC) are promising materials which have a variety of possible medical and dental applications. In situ setting and biocompatibility properties make CPCs potentially useful as endodontic filling materials, as implants for bony defects, and as a binder for other implant materials. CPCs are formed by moistening biphasic mixtures of calcium phosphate salts, usually anhydrous dicalcium phosphate (DCPA) and tetracalcium phosphate (TTCP), with limited amounts of water. Although relatively simple materials in composition, other chemical as well as physical properties, e.g. setting times, porosity and strength, are dependent in a complex manner upon a number of poorly understood parameters associated with the chemistry of the setting process. Particularly relevant are the solution parameters important in establishing the crystalline texture (i.e., size, shape, and aggregation properties) of the apatitic product formed upon completion of the DCPA/TTCP conversion, since the texture of this phase is a major determinant of the mechanical behavior of these cements. Thus, studies of solution influences on apatite crystal growth may prove useful in formulating cements with improved mechanical properties. Presently, the effect of solution supersaturation on apatite growth at pH 7.4 and 37°C is being examined. Results to date suggest that growth of individual apatite crystals is superseded by crystal proliferation as supersaturation increases. This transition from primary growth to secondary nucleation results in a more finely textured apatitic product.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00555-02 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Studies of the Structure and Function of PTS Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

D.A. Torchia, Chief, PBS, BRB, NIDR
J.G. Pelton, Senior Staff Fellow, BRB, NIDR
J.M. Schwab, Expert/Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

Johns Hopkins University

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.70	1.70	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Phosphoenolpyruvate:glycose phosphotransferase system (PTS) protein III^{Glc} was investigated by nuclear magnetic resonance (NMR) techniques in order to better understand the function of the protein. Studies were carried out to characterize (1) the three-dimensional (3D) molecular and chemical structure of phosphorylated III^{Glc} ($P\text{-}III^{Glc}$) and the interactions of III^{Glc} with the PTS phosphocarrier protein HPr.

Phosphorylation of III^{Glc} affects binding of the protein to sugar permeases, and thereby regulates the uptake of specific sugars by the cell. A regeneration system was developed that stabilized $P\text{-}III^{Glc}$ for many days, making it possible to show that the structure of $P\text{-}III^{Glc}$ was essentially identical to that of III^{Glc}. Hence, a change in protein electrostatics rather than structure is responsible for the different activities of III^{Glc} and $P\text{-}III^{Glc}$. In addition, the chemistry of the active site histidine residues was characterized in detail for III^{Glc} and $P\text{-}III^{Glc}$. The structure and active site chemistry were also elucidated for the H90Q variants of III^{Glc} and $P\text{-}III^{Glc}$. The results suggested that the interaction of H75 and H90 in $P\text{-}III^{Glc}$ facilitates phospho transfer. Finally, we have followed the interaction of III^{Glc} and $P\text{-}III^{Glc}$ with HPr by observing the changes in chemical shift of III^{Glc} amide groups that resulted when increasing levels of HPr were added to a solution of III^{Glc}. Although the interactions between the proteins appear to be weak, they nonetheless involve specific amino acid residues at the active sites of both proteins. The significance of this project lies in its potential for providing a rational quantitative understanding of the function of the PTS. The PTS has essential and diverse physiological roles in many bacterial cells, including those responsible for dental caries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00574-01 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amorphous Calcium Phosphate as an Inorganic Component in Dental Materials

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

E.D. Eanes, Chief, MCSS, BRB, NIDR
D. Skrtic, Visiting Associate, BRB, NIDR
A.W. Hailer, Chemist, BRB, NIDR

COOPERATING UNITS (if any)

Dental and Medical Materials Group, Polymers Division, NIST

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	1.3	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project, amorphous calcium phosphate (ACP), an important intermediate in the formation of apatite, is being investigated for possible use as a dental material. When either used alone, or in combination with other dental materials, especially polymeric resins, ACP has a wide range of possible applications such as in restorative composites, cavity liners and bases, luting and pulp capping agents, prophylactic and endodontic sealants, and as a component in periodontic packs and impression pastes. It has a number of potential advantages over other calcium phosphates for these purposes. As a dental cement, its advantage over current biphasic systems (e.g., dicalcium/tetracalcium phosphate mixtures) is its simpler, single solid phase formulation. When included as a component in appropriate resin-based composites, sealants and adhesives, ACP may be useful as a remineralization agent as well as a vehicle for sustained, controlled release of inorganic anticaries ions such as fluoride. Currently, chemical studies on various ACP-resin formulations are being carried out to examine ACP's potential as a remineralization agent. Results to date indicate that discs of ACP-embedded, methacrylate resins release calcium and phosphate ions at levels that exceed the thermodynamic minimum necessary for remineralizing damaged tooth surfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00575-01 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structural Studies of Profilin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

D.A. Torchia, Chief, PBS, BRB, NIDR
S. Archer, Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

Johns Hopkins University Medical School

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.7	.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Acanthamoeba profilin-1 is a 125 residue protein that binds to both actin and to the head groups of poly(phosphoinositide)s and may regulate both actin assembly and the phosphoinositide signaling pathway. In order to further the understanding of the activity of profilin at the molecular level we have determined its three dimensional structure using multi-dimensional heteronuclear NMR spectroscopy. A crystal structure of the protein has not yet been determined. The central feature of the profilin solution structure is a five stranded antiparallel beta sheet flanked by N- and C-terminal helices on one face, and by a third helix and a two stranded antiparallel beta sheet on the other face. Cross linking experiments and the location of conserved residues in profilins in different phyla suggest that actin binding occurs on the face of the protein occupied by the terminal helices. The other face of the molecule contains residues that may be important in determining the difference in polyphosphoinositide binding of profilin isoforms, suggesting that actin and lipid binding occur on adjacent faces of the protein. The significance of the project is that the profilin structure obtained provides the first information about the interactions of profilins with actin and lipids. These interactions are thought to link processes that regulate transmembrane signaling with formation of the cytoskeleton.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00576-01 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structural Studies of Fibronectin Modules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

D.A. Torchia, Chief, PBS, BRB, NIDR

V. Copié, Guest Researcher, BRB, NIDR

S. Akiyama, Senior Staff Fellow, LDB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fibronectin is a multidomain protein with a myriad of functions. Our goal is to determine the three dimensional structure of the domain consisting of the ninth and tenth type III modules. This domain contains the RGD cell attachment sequence and has full fibronectin binding activity. In order to obtain structures using NMR methods it has previously been necessary to work at protein concentrations of ca. 0.8mM or above. We have recently acquired a large volume NMR probe which allows us to work at concentrations of 0.4mM. We have worked out conditions that yield excellent NMR spectra at fibronectin domain concentrations of ca. 0.2-0.3mM. We are adjusting conditions so that it will be possible to obtain quality spectra at 0.4mM, and proceed with the structure determination.

The significance of the project is the insight that the structure of the RGD fibronectin domain will provide about the mechanisms of cell attachment and integrin recognition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00603-01 BRB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Bone Morphogenetic Proteins: Biological Significance of Their Redundancy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

S. Vukicevic, Visiting Scientist, BRB, NIDR
F.P. Luyten, Visiting Scientist, BRB, NIDR
P. Chen, Visiting Associate, BRB, NIDR
B. Hoang, Howard Hughes Fellow, BRB, NIDR

COOPERATING UNITS (if any)

School of Medicine, Zagreb, Croatia; ACTA, Faculty of Dentistry, Holland; Creative BioMolecules, Hopkinton, MA

LAB/BRANCH

Bone Research Branch

SECTION

Bone Cell Biology Unit, Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.4	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to define the role of bone morphogenetic proteins in cartilage and bone formation, both in embryogenesis and in postnatal life. Our discovery that several bone morphogenetic proteins are included in the process of chondrocyte maturation and endochondral bone formation raises the question of the biological significance of their redundancy. To further define the function of bone morphogenetic proteins 3 and 7 we performed localization studies during human and mouse development by northern and in situ hybridization and immunocytochemistry, utilizing specific riboprobes, polyclonal and monoclonal antibodies. Both BMP-3 and 7 showed a specific localization pattern and were coordinately expressed during chondrogenesis, osteogenesis and development of basement membranes. The presence of BMPs in basement membranes was further elaborated by performing in vitro binding studies. High affinity binding of BMP-3, 4 and 7, and TGF-β1 to collagen IV, suggested that type IV collagen may function as a TGF-β superfamily affinity matrix. Lung and kidney were detected as major sites of production of BMP 3 and 7 respectively, suggesting broader physiological functions as their involvement in calcium homeostases. In view of this we intend to analyze their in vitro function during the process of chondrocyte maturation and in vivo regulation in the kidney and skeletal tissues in animal models with rickets, hypercalcaemia and uremia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00212-17 CI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Taste and Its Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Weiffenbach, James	Research Psychologist	CIPC	NIDR
Baum, Bruce J.	Clin. Dir/Chief	CIPC	NIDR
Fox, Philip C.	Dental Offier	CIPC	NIDR
Ryba, Nicholas J.P.	Visiting Associate	CIPC	NIDR

COOPERATING UNITS *(if any)*

LSB, NIH; Audie L. Murphy Memorial Veterans Hospital, San Antonio, Texas
Bridget Krech, Normal Vol. Intern, SWD, CC
Pat Glines, Normal Vol. Intern, SWD, CC

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to elucidate the mechanisms by which oral perceptual experience is generated. Since objective measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, the routine assessment of taste is carried out using aqueous solutions representing each of the four basic tastes. Measures include both (detection) thresholds and judgments of intensity for taste stimuli at higher, more commonly encountered levels of strength. Olfactory function is routinely assessed by a standardized test of odor identification. Assessments of sensitivity to local pressure on the tongue and to variation in the temperature or the viscosity, of an oral bolus are also available. These methods, applied to the study of oral perceptual changes may occur with oral or systemic disease and its treatment, with salivary gland dysfunction, with aging or as an isolated complaint can provide insights into basic mechanisms of normal chemosensory perception. Direct investigations or oral sensory mechanisms in individuals lacking oral complaints or disfunction can also advance the understanding of the mechanisms by which the complex oral stimuli encountered in everyday life are perceived.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00332-12 CI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Investigations and Case Reports

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Guckes, Albert D.	Dep Clinical Director	CIPC	NIDR
Atkinson, Jane C.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chief	CIPC	NIDR
Brahim, Jaime S.	Senior Staff Dentist	CIPC	NIDR
McCarthy, George M.	Dental Officer	CIPC	NIDR
McCullagh, Linda	Clinical Nurse	CIPC	NIDR
Valdez, Ingrid	Dental Officer	CIPC	NIDR

COOPERATING UNITS *(if any)*

Lab. of Clin. Sci., NIMH; Ped. NCI; Inter-Inst. Genet. Prog.,CC, H.A. Brandt, Med. Ofc, LCS, HIMH, R.J. Elin, Chf,Clin.Path., CP, CC, J. Marini, Genet, CE, NCI, J.J. Mulvihill, Chf,Clin. Genet. Sect., CEN, CI, P.A. Pizzo, Chief, Ped. Br., POB, NCI

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.95	.80	1.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical case studies of unusual interest and clinically related research are being conducted on a variety of dentally related subjects. Research techniques being utilized include chart and literature reviews, and evaluation of various therapeutic regimens. The professional staff of the Patient Care and Clinical Studies Section, CIPCB, NIDR, are encouraged to become involved in clinically related research investigations and documentation of unusual cases. Interesting types of oral pathology with or without other medical complications are often seen in the NIDR Dental Clinic. Publication of these cases with a multi-disciplinary review of the disorder can provide valuable information for the dental clinican who may be required to treat similar conditions in the future. For example a recent project focused on oral changes asociated with HIV-1 infection in children. Particular attention has been paid to the high frequency of nursing bottle caries in the population. Other studies are related to research on endosseous dental implants (see Z01DE00412). For example we have documented the use of oral endosseous titanium implants to replace the mandibular teeth in Erdheim-Chester (E-C) disease and in Papillon-Lefevre syndrome, also referred to as generalized juvenile periodontosis. The use of endosseous implants in patients with these syndromes has been questioned because of the possibility of rapid bone loss around implants. Mandibular endosseous implants have successfully integrated in these patients. Further we have treated the maxillae of the patient with Papillon-Lefevre with a combination of bone grafting and endosseous implants.

Continuation of professional personnel)

ermillion, Cheryl	Dental Hygienist	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00336-12 CI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Salivary Gland Secretion Mechanisms During Normal and Altered Functional States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute

Baum, B.J.	Clin Dir/Chief	CIPC	NIDR
Ambudkar, I.	Senior Staff Fellow	CIPC	NIDR
Dai, Y.	Visiting Fellow	CIPC	NIDR
Hiramatsu, Y.	Visiting Associate	CIPC	NIDR
Lazowski, K.	Visiting Associate	CIPC	NIDR
Li, J.	Visiting Fellow	CIPC	NIDR
O'Connell, B.	Staff Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

PH, NHLBI, NIH; DNM, CC, NIH; Dept. of Nuclear Medicine, Univ. of Chicago; Dept. of Neurology, Cornell University

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Gene Transfer Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.97	4.47	1.50

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We utilize in vitro dispersed cells of salivary glands, in vivo cannulated glands, and cultured salivary cell lines as laboratory models to understand mechanisms controlling saliva formation. We have focused most of our studies on neurotransmitter regulation of secretory events. During this reporting period the primary focus of signaling studies continues to be muscarinic receptors (mAChRs) in rat parotid gland acinar cells. In parotid cells, stimulation of mAChRs results in the generation of inositol phosphates via the activation of phospholipase C. Subsequently this response leads to the elevation of cytosolic Ca^{2+} levels and fluid secretion. We have continued to characterize mAChRs in intact rat parotid cells using the binding of a subtype non-selective antagonist (NMS, N-methylscopolamine). We have determined that a moderate population (~30-40%) of spare receptors exist for inositol trisphosphate formation. We have continued in vivo studies of mAChRs in exocrine glands using iodinated QNB (quinuclidinyl benzilate) enantiomers and phamacokinetic analyses. These experiments have led to the development of a clinical research protocol to examine mAChRs in normal human volunteers. To understand how salivary glands transport water we have begun studies on a putative water channel, CHIP28. We have isolated a cDNA encoding a CHIP28-like protein from a rat parotid library and examined its cellular distribution in this gland by in situ hybridization. We have also initiated efforts to transfer foreign genes into rat salivary glands in vivo using replication deficient recombinant adenovirus (Ad) vectors (e.g. containing genes encoding E. Coli ß-galactosidase, ß gal; and human α 1 antitrypsin, α1AT). Two days after retrograde duct instillation ofAdß gal striking expression is seen in acinar and ductal cells of all major salivary glands. Transfer of the α1AT gene results in secretion of this protein in gland saliva for 4-10 days.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00337-12 CI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oral Physiological Processes: Normal Function and Disease Perturbation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Fox, Philip C.	Dental Officer	CIPC	NIDR
Adesanya, Margo	Dental Staff Fellow	CIPC	NIDR
Aladib, Walid	Visiting Fellow	CIPC	NIDR
Atkinson, Jane C.	Senior Staff Dentist	CIPC	NIDR
Baum, Bruce J.	Clinical Dir/Chief	CIPC	NIDR
Kurrasch, Regina	Expert	CIPC	NIDR
Macynski, Alice A.	Research Nurse	CIPC	NIDR

COOPERATING UNITS *(if any)*

RM, CC; DR, CC; LCI, NIAID; Columbia Univ., New York; Univ. of Utah; Univ. of California, San Francisco; Univ. of Colorado; Baylor Univ., Dallas; Univ. of Liverpool, Liverpool, England; Barbara C. Sonies, Speech Pathologist, RM, CC

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.60	5.60	1.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project examines the function of the salivary glands and other oral tissues in individuals with alterations of normal oral function due to disease or therapeutic procedures. Major efforts have been directed at the evaluation of patients complaining of xerostomia (oral dryness). Entry into all studies is through the Dry Mouth Clinic. Utilizing outpatient and inpatient services, specific evaluative and diagnostic approaches have been developed to aid in establishing the extent and causes of salivary gland dysfunction in the "dry mouth" patient. Major patient groups studied include individuals with Sjögren's syndrome, an autoimmune exocrinopathy, and those with salivary hypofunction secondary to therapeutic irradiation to the head and neck region. Oral and secretory effects of a number of other systemic diseases also are evaluated. Ongoing treatment protocols are evaluating the effectiveness of the parasympathomimetic drug pilocarpine for salivary stimulation in the post-radiation group and a combination of an anti-inflammatory drug and pilocarpine for Sjögren's syndrome patients. Clinical and laboratory studies focusing on the immunological basis of the salivary component of Sjören's syndrome have advanced and represent the main focus of our work. Markers of salivary gland disease activity in the serum of patients with Sjören's syndrome have been identified. The effects of cytokines and other immune mediators on a cultured human salivary cell line have been investigated. Work has begun on an *in vivo* animal model system for inflammatory salivary gland disease using an immunodeficient mouse strain.

Professional Personnel, continued

Valdez, Ingrid H.	Dental Officer	CIPC	NIDR
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR
Wu, Ava J.	Dental Staff Fellow	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00412-08 CI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oral Endosseous Titanium Implants in Edentulous and Ectodermal Dysplasia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brahim, Jaime S.	Senior Staff Dentist	CIPC	NIDR
Cooper, Lyndon F.	Staff Fellow	CIPC	NIDR
Guckes, Albert D.	Dep Clinical Director	CIPC	NIDR
McCarthy, George M.	Dental Officer	CIPC	NIDR
McCullagh, Linda	Clinical Nurse	CIPC	NIDR
Morgan, Victor L.	Dental Laboratory Technician	CIPC	NIDR
Folio, John	Consultant	CIPC	NIDR

COOPERATING UNITS *(if any)*

Rehab. Med. Dept., CC; Nutr. Dept., CC; Surg. Sv. Dept., CC; Diag. Systems Branch, NIDR; Com. Officers Dental Cl., CC; Eastman Dental Center, Nancy Siebring, Nutr. Res. Spec., NUTR, CC, Barbara C. Sonies, Speech Path., RM, CC

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.67	1.97	2.70

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project examines the use of endosseous dental implants in completely edentulous patients, pre and post adolescent patients with ectodermal dysplasia (ED) and multiple congenitally missing permanent teeth and adult patients requiring the replacement of single teeth. Removable dentures are considered a significant handicap related to mastication, speech, esthetics, reduction of the residual ridges of the mandible and maxillae, and body self image. Individuals with ectodermal dysplasia often have several congenitally missing teeth resulting in a lack of development of the alveolar bone which normally is present to support the permanent teeth. Lack of alveolar bone not only makes it difficult for a patient to wear a removable denture but also makes the placement of dental implants more difficult and possibly less successful. These studies are attempting to determine: (1) if dental implants can be used successfully to replace missing teeth in non-ED edentulous adult patients and adult and pre-adolescent patients with ectodermal dysplasia and multiple congenitally missing teeth; (2) if coating a titanium implant with hydroxyapatite improves its success when used to replace single missing teeth. Further, we are trying to assess if treatment with an implant supported fixed denture significantly affects loss of vertical dimension of occlusion, satisfaction with treatment, food choice and nutrition, perception of difficulty of chewing selected food, and body self image, when compared to treatment with a conventional removable denture. Also, the project is seeking to determine the effects of mandibular dental implants on the growth and development of the craniofacial complex of pre-adolescent patients with ED and hypodontia. Data from this project should provide information concerning the relationship of personality to body image and the ability to adapt to oral prostheses of various types. During this reporting period the laboratory investigations initiated to study the biology of the bone: implant interface entered a new phase with a recently initiated collaboration with the Bone Research Branch, focusing on methods of accelerating the initial healing process of bone surrounding newly placed implants and improving methods of bone grafting prior to placing implants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00415-08 CI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ion Transport and Fluid Secretion in Salivary Glands

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

Turner, Roy James	Visiting Scientist	CIPC	NIDR
Moore, Marilyn L.	IRTA Fellow	CIPC	NIDR
Moran, Arie	Visiting Associate	CIPC	NIDR
Valdez, Ingrid H.	Dental Staff Fellow	CIPC	NIDR
Reshkin, Stephen	Staff Fellow	CIPC	NIDR
Davis, Vincent	Dental Staff Fellow	CIPC	NIDR
Tanimura, Akihiko	Visiting Fellow	CIPC	NIDR

(See attached continuation sheet)

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.52	3.42	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Saliva is the principle protective agent for the mouth and thus is of primary importance to oral health maintenance. Perturbations of salivary secretory mechanisms can consequently lead to serious oral health problems. The objective of this project is to study the membrane and cellular processes which underlie the phenomenon of salivary fluid secretion and thus to contribute to our understanding of the fluid secretory process in normal and diseased states. Because similar secretory mechanisms are thought to be common to a number of other exocrine glands, this information should be of rather broad applicability and interest. During the present reporting period our specific areas of focus were the following.

(1) Studies of the regulation of the rat parotid acinar Na-K-2Cl cotransporter by secretagogues and other stimuli were continued.

(2) A polyclonal antibody against the rabbit parotid Na-K-2Cl cotransport protein was prepared and used to identify possible positive clones from a rabbit parotid cDNA library.

(3) Investigations of the functional properties of salivary ducts were continued. In particular, the properties of an amiloride-sensitive sodium channel were characterized.

(4) The effects of various attachment factors on the adhesion and spreading of the human salivary ductal cell line HSY were evaluated.

Continuation of professional personnel

Ferri, Concetta	Special Volunteer	CIPC	NIDR
Casavola, Valeria	Guest Worker	CIPC	NIDR
Evans, Richard	Visiting Fellow	CIPC	NIDR
Brookes, Neville	Guest Worker	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00438-07 CI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms Regulating Calcium Flux in Salivary Glands

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ambudkar, Indu S.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chief	CIPC	NIDR
Hiramatsu, Yukiharu	Visiting Associate	CIPC	NIDR
Lockwich, Timothy	Staff Fellow	CIPC	NIDR
Sawaki, Kohei	Visiting Associate	CIPC	NIDR
Kaplan, Mitch	Dental Staff Fellow	CIPC	NIDR
Hong, Irene	Dental Staff Fellow	CIPC	NIDR

COOPERATING UNITS *(if any)*

Department of Biological Chemistry, University of Maryland School of Medicine; Dept. of Medicine, Div. Nephrology, Johns' Hopkins Univ. School of Medicine; LB, NHLBI; LC, NIDDK;

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Secretory Physiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.85	4.60	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed towards understanding the processes which regulate cytosolic [Ca] in salivary gland cells. We are studying (i) receptor regulation of phosphatidylinositol bisphosphate-specific phospholipase C, (ii) regulation of Ca entry in salivary cells, and (iii) regulation of Ca flux in rat parotid gland basolateral membrane vesicles. Previously we have characterized a phosphatidyl-inositol-4,5,bisphosphate specific phospholipase C enzyme in rat parotid gland membranes. In this reporting period we have shown that it is independently regulated both muscarinic and α_1-adrenergic receptors via a mechanism mediated by α subunits of the $G_{q/11}$family of G-proteins. Sustained Ca entry into parotid acini maintains a sustained elevation of Ca in the cytosol and thus facilitates prolonged fluid secretion. While we have earlier shown that Ca entry is correlated with the depletion of Ca from intracellular Ca stores, the exact mechanism which regulates this process is yet unclear. Using Mn as a substitute divalent cation, we have now shown that Ca entry into internal Ca pool depleted cells, via agonist stimulation, is very sensitive to temperature. Additionally, the pattern of its response to temperature distinguishes it from Mn entry into unstimulated acini. In a human salivary gland cell line, HSG, we have identified a Ca entry mechanism which is not regulated by the emptying of intracellular Ca stores, but depends on muscarinic receptor activation of G-protein(s). Consistent with our date with intact acini we have observed that Ca influx into isolated basolateral membrane vesicles is decreased at low temperature. We have observed that low temperature also protects Ca influx in vesicles against inactivation by trypsin and carbodiimides, which suggests that temperature induces a modification of the Ca influx pathway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00458-06 CI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

β-Adrenoreceptors and Gene Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute

Kousvelari, Eleni	Expert	CIPC	NIDR
Ambudkar, Indu S.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chief	CIPC	NIDR
Chiaki, Kitamura	Special Volunteer	CIPC	NIDR
Lazowski, Krzysztof W.	Visiting Associate	CIPC	NIDR
Mertz, Prema M.	Staff Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

Department of Pharmacology, University of Minnesota; VA Medical Center

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Secretory Physiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.01	2.76	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The studies presented here are designed to; (i) examine the timing of appearance of the extracellular matrix (ECM) components, laminin, collagen IV and I and their receptors of the integrin family during rat parotid gland development; (ii) isolate and characterize the gene encoding a proline-rich protein (PRP); (iii) compare the timing of appearance of ß-adrenergic receptors (ß-AR) with the PRP gene expression during development; and (iv) investigate the role of laminin and collagen IV on the A5 cell adherence. During this reporting period we have; (1) shown that laminin B1, B2 but not A chain, collagen IV and I genes and α6 and ß1 integrin genes are highly expressed during the early stages (0, 7 and 14 days) of parotid gland development and decline to the adult levels at 21 days. High levels of laminin, collagen IV and ß1 integrin were localized in the basal membrane around the developing acinar and ductal cells and lower amounts at later times. This pattern of expression is concomitant with the morphological and proliferative changes occurring in these cells during development; (2) isolated four PRP related clones and characterized one of these clones, PRP 5. This PRP 5 gene contains a putative cAMP regulatory element in the 5' flanking region; (3) demonstrated that the high levels of ß-AR at 21 days of age corresponds with the high levels of the PRP mRNA at this time; (4) shown that laminin and/or collagen IV promote the early adherence of A5 cells in a focal like pattern.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00049-22 LCDO

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Function of Transglutaminases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Chung, S.I.	Research Chemist	LCDO, NIDR
Others:	Kwon, S.W.	Visiting Fellow	LCDO, NIDR
	Hwang, H.K.	Guest Researcher	LCDO, NIDR
	Folk, J.E.	Chief, EC Section	LCDO, NIDR

COOPERATING UNITS *(if any)*

Peter Steinert, Laboratory of Skin Biology, NIAMSD
Soo Yeol Kim, Laboratory of Skin Biology, NIAMSD
Franco Carmassi, 2nd Medical Clinic, Pisa University, Pisa, Italy

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD 20892-0030

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.66	2.5	.16

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The crosslinking reaction, the extent of which affects the structural integrity of the epithelium, is catalyzed by transglutaminases (TGases) through the formation of Nε(γ-glutamyl) lysine isopeptide bonds. An understanding of the molecular features of these enzyme, their cellular regulation and identification of their protein substrates in the body epidermis and in mucosal epithelial cells will provide insight into the physiological role of the crosslinking process.

Expression of TGase K and E and their variant mutant clones in E. coli has provided a means of determining structural requirement for generation of maximum catalytic activity. In each enzyme, there is a highly conserved sequence of amino acids essential for enzyme activity. In TGase K, an amino-terminal peptide, residues 1-57, regulates activation of the enzyme. Cleavage at residue 570 provides maximum catalytic activity. In TGase E, maximum activity is obtained by cleavage at residue 471. In both enzymes, a C-terminal peptide influences substrate specificity. Limited proteolysis of both enzymes by Ca++-dependent protease appears to be the source of regulation in differentiating keratinocytes.

Blood clots from a tuberculosis patient on isoniazid treatment with hemorrhagic symptoms were rapidly lysed by plasmin. The plasma of this patient contained normal factor XIII levels but no crosslinking of fibrin occurred even though the isolated fibrinogen formed normal crosslinked fibrin clots. An IgG fraction from this plasma was found to be responsible for the inhibition of crosslinking. The basis of this unique and potentially important anti-body reaction is under further study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00311-13 LCDO

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hypusine in eIF-4D: Biosynthesis and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Park, M.H.	Research Chemist	LCDO, NIDR
OTHERS:	Folk, J.E.	Chemist	LCDO, NIDR
	Wolff, E.C.	Expert	LCDO, NIDR
	Joe, Y.A.	Visiting Fellow	LCDO, NIDR
	Lee, Y.B.	Visiting Fellow	LCDO, NIDR

COOPERATING UNITS *(if any)*

Dr. H. Hanauske-Abel, Cornell University, Medical College, New York, NY; Dr. Marc Lalande, Harvard Medical School, Boston, MA; Dr. Andy Karplus, Cornell University Ithaca, NY.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.25	4.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Eukaryotic protein translation iniation factor 5A (eIF-5A) contains one residue of hypusine and appears to be the only cellular protein with this unique amino acid. Hypusine is produced post-translationally by transfer of the butylamine portion of the polyamine spermidine to a lysine residue in the eIF-5A precursor to form deoxyhypusine followed by hydroxylation to form hypusine. These findings reveal a novel cellular metabolic pathway. Hypusine is essential for the biological activity of eIF-5A in an in vitro translation initiation assay and hypusine and eIF-5A appear to be vital elements for growth of eukaryotic cells. Thus, the hypusine biosynthetic steps, deoxyhypusine synthesis and deoxyhypusine hydroxylation present special potential targets for intervention in cellular proliferation. Several inhibitors of the enzymes deoxyhypusine synthase, and deoxyhypusine hydroxylase were developed and their cellular effects have been examined. The most potent inhibitor of deoxyhypusine synthase monoguanyl-1,7-diaminoheptane, (Ki=0.01μM compared to the Km of spermidine of 4μM) in vitro is the most effective inhibitor of hypusine formation and growth in CHO cells (IC 50 for growth inhibition 0.3μM). The compound is taken up actively by the polyamine transport system. Mutant CHO cells defective in polyamine transport are resistant to growth inhibition by monoguanyl-1,7-diaminoheptane indicating that it exerts antiproliferative activity intracellularly by inhibition of hypusine synthesis. The guanylated diamines represent a new class of antiproliferative agents, the action of which is targeted toward a single specific cellular process, hypusine biosynthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00433-07 LCDO

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional aspects of C-reactive protein.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robey, F.A.	Chief, PIU	LCDO, NIDR
OTHERS:	Heegaard, N.	Visiting Fellow	LCDO, NIDR
	Ivanov, B.	Visiting Fellow	LCDO, NIDR
	Liu, M.	Visiting Fellow	LCDO, NIDR

COOPERATING UNITS *(if any)*

Henry Gewurz, Rush Medical School, Chicago.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

C-Reactive protein (CRP) and serum amyloid P component (SAP) are two closely related proteins with respect to their primary structure and their pentameric appearance under the electron microscope. The two proteins have unknown functions. A common property shared by CRP and SAP is their ability to bind to sulfated polysaccharides and to fibronectin in a calcium-dependent manner.

Using techniques including cell attachment assays, tissue culture, peptide synthesis and immunoassay, a peptide modeled after the primary sequence of SAP was found to bind strongly and specifically to heparin and certain other sulfated polysaccharides. This binding was independent of calcium. The homologous peptide from CRP also bound heparin.

Using capillary zone electrophoresis we were able to demonstrate for the first time the binding of a small peptide, F-T-L-C-F-R tp a simple sugar, mannose-6-phosphate. This interaction occurs at any pH and is dependent of divalent cations such as Ca2+ Both, SAP and CRP will bind to heparin-Sepharose at pH 5.5, the pH of tissue during inflammation. This pH is independent of Ca2+ and clearly parallels the synthetic peptide. This finding points to a role for the CRP-heparin complex during inflammation at lower pH values where CRP was previously shown to activate serum complement. In addition, the ability to bind DNA and activate complement at low pH might be indicative of a role played by CRP in the transfer of genetic material during tissue destruction. Combined, these results point to a more complete understanding of the acute of the acute phase response. Such knowledge will be useful in developing therapeutics and vaccines whose unknown side effects include the induction of the acute phase response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00434-07 LCDO

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on HIV-1 Targeted Drug Delivery Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robey, F.A.	Chief, PIU	LCDO, NIDR
OTHERS:			
	Harris-Kelson, T.	Staff Fellow	LCDO, NIDR
	Ivanov, B.	Visiting Fellow	LCDO, NIDR

COOPERATING UNITS *(if any)*

Marjorie Roberts-Guroff, National Cancer Institute; Seth Pincus NIAID, Peter Roller, NCI; Mario Cleriqi, NCI; Marian Neutra, Harvard University; Peter Nara, NCI

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.75	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

HIV -1 is the causative agent of AIDS. CD4 is the cellular receptor for HIV-1 and its amino acid sequence is known. The region of HIV-1 that binds to CD-4 is termed gp160 and this is the envelope glycoprotein that is composed of gp120 and gp41. The gp120 region specifically binds to CD4 and the sequences of amino acids in both CD4 and gp120 that are responsible for the high affinity binding of the virus are now known.

Prior to this report, it was believed that the binding site for gp120 on CD-4 could not be chemically synthesized. The reason for this is that the binding site is believed to be conformationally constrained.

We have synthesized a peptomer from amino acids 419-436 of the gp120 from the MN isolate of HIV-1 and we have learned that, by specifically polymerizing peptide (419-436) we enhance the α-helical conformation of the peptide. With peptomer (419-436), we have learned the following: First, 96% of the patients suffering from HIV-1 infection have antibodies to this region of gp 120 and these antibodies are able to neutralize HIV-1 infection in vitro. Second, peptomer (419-436) binds CD4 and this binding inhibits human antibodies from binding peptomer (419-436). In addition, CD4-expressing cells such as CEM and MOLT-3 bind peptomer (419-436).

Third, rabbit antibodies produced in Freund's adjuvant against peptomer (419-436) do not recognize gp120 or neutralize HIV-1 infection in vitro. However, rabbit antibodies produced in Ribi's adjuvant do recognize gp120 and they do neutralize HIV-1 infection in vitro. These results indicate that the binding of HIV-1 to CD4 is conformation-dependent and future therapeutics and vaccines that target this binding must be developed with conserved conformational constraints.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00479-05 LCDO

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms Responsible for Oncogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robbins, K.C.	Chief, MCBS	LCDO, NIDR
Others:	Matoskova, B.	Visiting Fellow	LCDO, NIDR
	Marcilla-Diaz, A.	Special Volunteer	LCDO, NIDR
	Rivero, O.	Visiting Fellow	LCDO, NIDR

COOPERATING UNITS *(if any)*

Joseph B. Bolen, Bristol Meyers Squibb, William J. LaRochelle, LCMB, NCI; Timothy J. Ley, Washington University, St. Louis, Missouri, Oliver A. Sartor, LCDM, NCI

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.86	2.20	.66

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three approaches were taken to address the mechanism of cellular transformation induced by nonreceptor protein-tyrosine kinases. One involves overexpression of fgr genes specifying normal or aberrant kinases in NIH/3T3 cells. Our findings document a rare malignant transformation by high levels of p55-c-fgr. Furthermore, it was shown that by mutation of sequences encoding the carboxyl terminus of p55-c-fgr the c-fgr gene is converted into a potent, dominant acting oncogene. A search for substrates for these activated tyrosine kinases has identified molecules of 135 kd and 70 kd that preferentially interact with and are tyrosine phosphorylated by transforming as compared to normal versions of src, fyn, and fgr kinases. The third approach involves searching for evidence of activated tyrosine kinases in naturally occurring human neoplasia, especially squamous cell carcinomas of the head and neck. We have found that the receptor for epidermal growth factor is activated in a majority of oral squamous cell carcinomas and have identified a novel mechanism for activation of this receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00480-05 LCDO

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Normal Physiologic Roles for Nonreceptor Protein-Tyrosine Kinases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robbins, K.C.	Chief, MCBS	LCDO, NIDR
Others:	Gutkind,J.S.	Visiting Fellow	LCDO, NIDR
	Agarwal, A.	Visiting Fellow	LCDO, NIDR
	Salem, P	Visiting Fellow	LCDO, NIDR
	Rivero, O.	Visiting Fellow	LCDO, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.41	2.75	.66

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our working hypothesis states that nonreceptor protein-tyrosine kinases transduce environmental signals in fully mature cells. During the current reporting period important evidence has been obtained supporting this hypothesis.

Previous studies have demonstrated that multichain immune recognition receptors, such as the T-cell receptor, signal their occupancy by inducing tyrosine phosphorylation of cellular protein substrates. Type I and II receptors for the Fc portion of IgG are single chain immune recognition receptors having external, transmembrane and cytoplasmic domains. In the present study, we have investigated the possibility that upon engagement, Fcγ receptors induce protein-tyrosine phosphorylation. Our findings reveal increased phosphorylation of a number of proteins on tyrosine residues after crosslinking of either high (FcγRI) or low (FcγRII) affinity receptors expressed on HL60 cells. Engagement of Fc RII induced rapid tyrosine phosphorylation that decayed to basal levels by 40 min. In contrast, phosphorylation induced by FcγRI crosslinking was more delayed, peaking at 5-10 min and returning to basal levels by 60 min. Kinase assays of cellular proteins immunoprecipitated from lysates of activated cells by antibody to phosphotyrosine revealed phosphorylation of a 72 kDa molecule that was not present in lysates of resting cells. This phosphoprotein was identified as p72syk by immunoprecipitation with antibodies directed against two different regions of the syk gene product. Immunoprecipitation with antibodies directed p72syk followed by immunoblotting with anti-phosphotyrosine antibodies revealed an activation dependent tyrosine phosphorylation of p72syk. Thus, our present findings demonstrate induction of protein-tyrosine phosphorylation following engagement of monomeric immune recognition receptors and identify p72syk as a tyrosine kinase substrate involved in signalling by FcγRI and Fc RII.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00551-02 LCDO

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of G Proteins in Growth Control and Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gutkind, J.S.	Chief, MSU	LCDO, NIDR
OTHERS:	Robbins, K.C.	Chief, LCDO	LCDO, NIDR
	Xu, N.	Visiting Associate	LCDO, NIDR
	Coso, O.	Visiting Fellow	LCDO, NIDR
	Glasper, Y.	Special Volunteer	LCDO, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular Signalling Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.75	3.0	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of the project is to study the role of heterotrimeric G proteins and their coupled receptors in normal cell growth and oncogenesis. We have genetically engineered NIH 3T3 mouse fibroblasts to express the family of human acetylcholine muscarinic receptors (mAChRs). Using this model, we have shown that genes for mAChRs subtypes coupled to the activation of phosphatidylinositol (PI) hydrolysis can act as ligand-dependent oncogenes, whereas those coupled to the inhibition of the adenylyl cyclase (AC) are not. We have studied the role of Raf-1 in mitogenesis and cellular transformation induced by G protein-coupled receptors. Our findings suggest that the Raf-1 kinase plays a critical role in transformation induced by this class of receptors, however these cell-surface receptors might also utilize signalling routes bypassing the requirement of Raf-1. We have observed that growth promoting pathways activated by receptors coupled to G proteins involve tyrosine phosphorylation of a small set of cellular proteins previously identified as substrates for oncogene-encoded tyrosine kinases, such as the $p125^{FAK}$ and p130 v-*src* substrates. We have studied the relationship between *ras*-GAP and G protein coupled receptors. Cotransfection of wild-type GAP prevented transformation by m1 mAChRs, whereas the mutant consisting of only its catalytic domain lacked any demonstrable effect. In contrast, the N-terminal non-catalytic domain of GAP effectively prevented m1-induced focus-formation. Thus, our findings suggest a role for the N-terminal non-catalytic domain of GAP in regulating biological functions mediated by G protein-coupled receptors. The recent discovery of a new family of G proteins, G_{12}, distantly related to those that regulate the activity of adenylyl cyclases or PI-PLC prompted us to ask whether this novel class of G proteins harbors oncogenic potential. Our results show that, in contrast to α_{i2} and α_q, overexpression of wild type α_{12} in NIH 3T3 cells is itself weakly transforming, and mutationally activated α_{12} behaves as one of the most potent oncogenes described to date. Transformation does not involve PI-PLC but correlates with alterations in other G protein-linked pathways. In addition, a systematic screen of tumor-derived cell lines suggests that alterations in the expression of this G protein might contribute to human neoplasia, particularly in adenocarcinoma of the breast and salivary glands.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00558-02 LCDO

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oral Carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robbins, K.C.	Chief, MCBS	LCDO, NIDR
OTHERS:			
	Cardinali, M.	Visiting Associate	LCDO, NIDR
	Jakus, J.	Visiting Fellow	LCDO, NIDR

COOPERATING UNITS *(if any)*

John Ensley, Wayne State University

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.01	2.35	.66

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have approached oral carcinogenesis by initially searching for evidence of activated tyrosine kinases in naturally occurring human neoplasia, especially squamous cell carcinomas of the head and neck. Initial results have shown that the receptor for epidermal growth factor (EGF) is activated in a number of oral cavity tumors. As a probe for the mechanism of this activation, we have investigated the effect of suramin treatment on EGF receptor activity. It was expected that suramin would greatly reduce the level of intracellular protein-tyrosine phosphorylation by the EGF receptor. Instead, the drug dramatically enhanced protein-tyrosine phosphorylation in epithelial cell tumors. The mechanism at play was shown to involve activation of the growth factor, namely transforming growth factor alpha, that in turn stimulates the tyrosine kinase activity of the EGF receptor. These results suggested that suramin may stimulate the growth of certain tumors, and indeed suramin enhances the growth of oral carcinoma cells in culture. Have also investigated the possible involvement of tyrosine kinases in the development and maintenance of squamous cell carcinomas (SCC) of the upper aerodigestive tract, specifically asking whether aberrant protein-tyrosine phosphorylation could be observed in these tumors. A panel of 19 cell lines established at Wayne State University (WSU) from primary and metastatic SCC of the head and neck were evaluated. Utilizing assays the are capable of scoring EGFR activities, we have found evidence for enhanced EGFR action in 15 of the lines. Futhermore, with respect to normal human keratinocytes each of the 19 cell lines displayed excessive levels of tyrosine phosphorylation of cellular proteins. These results suggest a much broader contribution of the EGFR to naturally occurring carcinogenesis than previously appreciated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00230-17 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Proteins in Tissue Architecture and Cell Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kleinman HK	Section Chief	LDB, NIDR
Others:	Kibbey MC	Staff Fellow	LDB, NIDR
	Grant DS	Visiting Associate	LDB, NIDR
	Weeks BS	NRSA Fellow	LDB, NIDR
	Schnaper HW	Special Expert	LDB, NIDR
	Cid, M	Guest Researcher	LDB, NIDR

COOPERATING UNITS *(if any)*

NIA (Jucker M); NIAID (Fauci A); Johns Hopkins Med Sch, Balt MD (Walker L); Georgetown U Med Sch Wash DC (Dym M); Yale U Med Sch (Rosen E); U Wisconsin Med Sch (Auerbach R); Harvard Univ Boston MA (Neve R).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.15	3.06	1.09

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The extracellular matrix has been found to be important in embryogenesis and in repair. From in vitro studies using purified components, a better understanding of how cells adhere, migrate, proliferate, and differentiate in response to tissue and cell-specific matrix molecules has been established. We have found that the basement membrane, the extracellular matrix which underlies all epithelial cells and endothelial cells and surrounds nerve cells, promotes cell differentiation in vitro. When cultured on basement membrane, endothelial cells form capillary-like structures with a lumen, bone cells form canaliculi, salivary cells form glands, etc. Our goal is to define the molecular and cellular events involved in this process. Our approach has been to identify the (1) biologically active matrix components, (2) localize active sites on the matrix component with site specific antibodies and synthetic peptides, (3) identify and characterize cellular receptors, (4) gain an understanding of the intracellular events involved in the biological response, and (5) identify genes induced by the extracellular matrix. Specifically, we have used the endothelial cell tube forming assay to identify angiogenic factors including scatter factor (hepatocyte growth factor), haptoglobin (which is elevated in vasculitis patients), and estrogens. Estrogens have been found to promote leukocyte adhesion to endothelial cell monolayers via an increase in endothelial cell selectin adhesion receptors. This finding may explain the increase in inflammatory diseases in women. In addition, estrogens promote endothelial cell adhesion, growth and migration. Using the endothelial cell tube assay, a new role for proteases has been defined and may have important clinical uses in vessel repair. Subtractive cDNA cloning of endothelial cells on plastic vs basement membrane has identified several novel genes as well as thymosin B4 and calmodulin as induced during differentiation into vessels. The laminin-derived peptide SIKVAV promotes neurite outgrowth. A brain derived cellular receptor for SIKVAV shares homology with the amyloid precursor protein and may define the role of this protein in development and in Alzheimer's disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00481-05 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Connective Tissue Gene Expression in Development and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Klotman PE	Special Expert	LDB, NIDR
Others:	Bruggeman LA	Staff Fellow	LOM, NIDR
	Ray PE	IPA	LOM, NIDR
	Kopp JB	Sr Research Invest	LOM, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Chemoprevention, NCI (Roberts A, Sporn M); University of Maryland, Baltimore MD (Hansen B); Laboratory of Experimental Carcinogesis, NCI (Thorgeirsson S).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Disease Pathogenesis Group

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of these studies is to determine the molecular mechanisms responsible for the regulation of extracellular matrix proteins, to explore inducible factors that modulate mitogenesis and cellular matrix protein production such as hormones, autacoids, cytokines, and growth factors, and to develop novel therapeutic strategies to prevent progressive fibrosis and sclerosis. To accomplish these goals, we have focused on the normal regulation of the extracellular matrix during nephrogenesis and the mechanisms responsible for abnormal metabolism of extracellular matrix in renal disease states. During renal development, basement membrane genes are expressed in a coordinated fashion in different portions of the nephron. After nephrogenesis is complete and during adult life, matrix proteins are synthesized at low levels, presumably representing on-going replacement and remodeling of basement membrane and mesangial matrix. A common feature of the renal response to metabolic injury or inflammation is increased deposition of extracellular matrix proteins. The kidney is exquisitely sensitive to this process since normal renal function is predicated on a precise structure-function relationship. Thus, scarring anywhere along the nephron threatens the overall filtration process. Recent studies have 1. uncovered a novel role for products of arachidonic acid metabolism in the regulation of the renal mesangial matrix, 2. defined a new interrelationship for TGF-β2 and the renin-angiotensin system, 3. defined the cis-acting elements within the promoter of the TGF-β2 gene, and 4.demonstrated an important role for TGF-β in the regulation of metalloproteinases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00482-05 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tumor Growth and Metastases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kleinman HK	Section Chief	LDB, NIDR
Others:	Yamamura K	Visiting Fellow	LDB, NIDR
	Kibbey MC	Staff Fellow	LDB, NIDR
	Grant DS	Visiting Associate	LDB, NIDR
	Jun SH	Guest Researcher	LDB, NIDR
	Kim WH	Guest Researcher	LDB, NIDR

COOPERATING UNITS *(if any)*

UCSF, San Francisco, CA (Kim Y); NIA, (Passaniti A); Developmental Biology Center, University Wisconsin, Madison, WI (Auerbach R); NCI (Yanelli Y); Lombardi Cancer Center, Wash, DC (Thompson E); NINDS (Oldfield E).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.22	3.05	0.17

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies are conducted to define the mechanisms involved in tumor growth and metastasis and to develop new animal models of human cancers. We have found that a basement membrane extract (Matrigel) when premixed with human tumor cells (which do not grow well in mice) promotes their incidence and growth. Very low cell numbers can be used. We have been able to culture new highly differentiated human tumor cell lines from the tumors grown in mice including certain colon cell lines and a Nelson's pituitary tumor cell line. Laminin, a major basement membrane component, has been found to promote the malignant phenotype. Various biologically active laminin-derived synthetic peptides have been identified. YIGSR from the B1 chain blocks lung colonization, reduces tumor growth, and inhibits angiogenesis. Using multiple cycles of YIGSR adhesion, YIGSR adherent melanoma cells were derived and formed more tumors in lung colony assays and larger tumors in the subcutaneous model than the parent cells. The YIGSR non-adherent cells formed few tumors. Selection for adhesion to laminin was carried out with a human colon cancer and the adherent cells were found to be highly malignant when injected intracecally with Matrigel. These cells grew well and metastasized to the liver and surrounding tissues. This progression models the pathological process in humans. Another laminin-derived peptide containing SIKVAV from the A chain has been found to increase tumor growth, lung colonization, and angiogenesis as well as collagenase IV activity and plasminogen activation. This peptide was found to promote angiogenesis in one in vivo model by increasing the recruitment of neutrophils. Using this information and the newly developed models of human tumors, the development of new therapeutic strategies for cancer should be facilitated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00483-05 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Regulation and Function of Cartilage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yamada Y	Section Chief	LDB, NIDR
Others:	Matsuki U	Visiting Fellow	LDB, NIDR
	Hatano O	Visiting Fellow	LDB, NIDR
	Gao L	Visiting Fellow	LDB, NIDR
	Rhodes C	Biologist	LDB, NIDR
	Hayes N	Biologist	LDB, NIDR

COOPERATING UNITS *(if any)*

Shriner's Hospital (Doege); Johns Hopkins University (Francomano C); Aichi Medical University (Kimata K); Wistar Institute (Caton CA); University of Tennessee (Yoo TJ).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.09	3.09	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to understand the mechanisms underlying cartilage and craniofacial development. We have studied gene defects in diseases with skeletal and craniofacial abnormalities and identified genes involved in normal development of cartilage and cranofacial tissues. Mouse cartilage matrix deficiency (cmd) is an autosomal recessive mutation characterized by cleft palate, short limbs, tail and snout, and homozygous mice dye just after birth. Cartilage from cmd mice has normal levels of collagen II and link protein, but lacks aggrecan. We mapped the aggrecan gene close to mouse chromosome 7 to which cmd had been mapped. The levels of mRNA for aggrecan from cmd mice was low compared to that from normal mice. We identified a 7 bp deletion in exon 5 of the aggrecan gene of the cmd mice resulting a truncated molecule. We found a new enhancer sequence in the first intron of the collagen II gene which increased the promoter activity of the gene in chondrocytes. The minimum size of the enhancer was about 100 bp and contained a sequence homologous to a sequence in the promoter region of the link protein gene. Gel retardation analysis suggested that the promoter and the enhancer of the collagen II gene could form a DNA-loop structure by interacting with multiple nuclear factors. Glucocorticoid responsive element was identified in the first intron of the link protein. A segment between -920 and -750 bp of the promoter of the link protein was found to increase transcriptional activity of the gene. This segment contained a sequence homologous to a portion of the enhancer of the collagen II gene suggesting that a common nuclear factor was involved in the coordinate regulation of the collagen II and link, protein genes in chondrocytes. We have initiated a genome project to identify novel genes which regulate craniofacial and tooth development. Two cDNA libraries were constructed using mRNA from mouse embryo maxillofacial tissues and rat incisor pulp tissues. About 400 cDNA clones from each library were sequenced. We have been characterizing these clones by examining their stage- and tissue-specific expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00484-05 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Animal Models of Connective Tissue Disease in Transgenic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yamada Y	Section Chief	LDB, NIDR
Others:	Yamada K	Chief	LDB, NIDR
	Watanabe H	Visiting Fellow	LDB, NIDR
	Strong D	Bio Lab Tech	LDB, NIDR
	Gabriel V	Bio Lab Tech	LDB, NIDR

COOPERATING UNITS *(if any)*

Shriner's Hospital (Doege K); Osaka University (Kimura T); Wistar Institute (Caton GA); HSP Research Institute (Yanagi H); Mark Sharp & Fohme Research Laboratories (Hutchinson NI); DNX (Grass DS); Veterans Administratrion (Teckeltaub R).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.65	1.40	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Creation of gene targeted mutations and introduction of dominant mutations in transgenic mice have proved to be useful for understanding functions of proteins in development and as animal models for therapeutic applications for various diseases. The purpose of this project is to create transgenic mice for studying the molecular basis of genetic and acquired diseases associated with connective tissues as well as development. Genes for basement membrane and cartilage components have been cloned and the exon-intron structure of these genes have been characterized. Creation of transgenic mice with mutated exogenous genes for collagen IV and laminin chains have been exploited. Constructs for expression of foreign genes in cartilage in transgenic mice under the control of the promoter and enhancer of collagen II gene have been prepared to create animal models for human diseases such as arthritis and diabetes. Constructs containing reporter genes under the direction of promoters of the genes for collagen II and IV have been prepared and injected into mouse oocytes to identify sequences necessary for tissue specific expression. The creation of mutations in the endogenous genes for basement membrane and cartilage proteins has been attempted by homologous recombination. By using gene targeting in embryonic stem cells, mutations of specific sites within the genes can be introduced in the mouse germ line to assess the role of these genes in the whole animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00485-05 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Regulation and Function of Basement Membrane

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Burbelo PD	Senior Staff Fellow	LDB, NIDR
Others:	Yamada Y	Chief	LDB. NIDR
	Utani A	Visiting Fellow	LDB, NIDR
	Nomizu M	Visiting Associate	LDB, NIDR
	Shibuya M	NCI-JFCR	LDB, NIDR
	Sugiyama S	Visiting Associate	LDB, NIDR
	Takami H	Visiting Fellow	LDB, NIDR

COOPERATING UNITS *(if any)*

NINCDS (Wujek J, Kedar V); Max-Plank-Institute (Timpl R); Univ. of Pittsburgh (Hassell J); Univ of Genova (Noonan D); MD Anderson Cancer Center (Carson D); INSERM U49 (Clement B).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.97	4.97	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basement membranes are thin sheets of extracellular matrix surrounding most tissues and provide scaffolding for cells, filter proteins in tissues, and regulate cell growth and differentiation. Basement membranes consist of a unique set of proteins, including collagen IV, laminin, perlecan and nidogen/entactin. The α1(IV) and α2(IV) genes are separated by only 130 bp in a head-to-head orientation. Two enhancers, E-A and E-B were identified. E-A activated the promoter activity of both α1(IV) and α2(IV) genes, whereas E-B activated only α1(IV) promoter. A DNA binding protein was identified by cDNA cloning which bound to the bidirectional promoter. This protein is likely the large subunit of DNA replication complex A1 suggesting a dual function of the protein for DNA replication and transcription of collagen IV genes. Laminin is a family of heteromeric glycoproteins specific in basement membranes. A short unique sequence necessary for trimer assembly was identified at the C-terminal portion of the long arm of each of the laminin chains. An in vitro reconstitution assay using recombinant laminin chains revealed that only certain combinations of laminin chains were capable of forming trimers and dimers. Charged amino acid residues within the assembly sites of each of the chains were found to be critically important for chain-specific assembly suggesting that interchain ionic interactions determine laminin chain recognition. Thermal stability of these trimers and dimers were analyzed by CD spectroscopy. The inhibition of tumor cell metastasis by the laminin B1 chain YIGSR peptide was found to be increased by multimerization of the peptide through lysine conjugation. Nine new cell adhesion sites were identified in the G domain of the laminin A chain by a synthetic peptide approach.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00508-04 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Human Immunodeficiency Virus I (HIV-1)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Klotman PE	Special Expert	LDB, NIDR
Others:	Kopp JB	Sr Research Invest	LOM, NIDR
	Weeks BS	NRSA Fellow	LDB, NIDR
	Adler SH	Guest Researcher	LDB, NIDR
	Ray PE	IPA	LOM, NIDR
	Rappaport J	Staff Fellow	LOM, NIDR
	Bruggeman LA	Staff Fellow	LOM, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Tumor Cell Biology, NCI (Klotman M).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Disease Pathogenesis Group

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.05	1.55	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of these studies is to explore the role of extracellular matrix proteins and their receptors in the pathogenesis of AIDS. Recent work has focused on HIV-associated nephropathy which is increasingly recognized as a complication of infection with HIV-1, particularly among African Americans. Although the pathology has been well-characterized, the mechanisms by which HIV induces renal disease remains largely unknown. To address this issue, we have established a transgenic mouse model using HIV-1 proviral DNA rendered non-infectious by a 3 kb deletion overlapping the gag and pol genes. This construct contains the viral LTRs and encodes envelope glycoproteins as well as regulatory and accessory genes. Heterozygous mice develop renal disease essentially identical to HIV associated nephropathy in man with focal segmental glomerulosclerosis and tubulointerstitial disease. These findings support an important role for viral proteins in AIDS pathogenesis. As a result, we are currently exploring whether renal cells can sustain productive infection and how HIV-1 infected cells can target renal tissues. We have found that infected cells express surface adhesion molecules that recognize endothelium and basement membrane proteins. We have also found that T-cells and macrophages infected with HIV-1 express cell surface adhesion receptors, attach to basement membranes, and release proteases that facilitate tissue invasion. Current studies are directed to the development of new HIV-1 transgenic lines with single genes under the control of tissue-specific promoters, the testing of gene therapeutic constructs in our current transgenic lines, and new strategies for therapy including systemic antisense and HIV-1-targeted ribozymes.

"Professional Personnel, continued"

Bryant J	Veterinarian	ACU, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00524-03 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functions and Developmental Regulation of Matrix Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yamada KM	Chief	LDB, NIDR
Others:	Brown KE	IRTA Fellow	LDB, NIDR
	Yamada S	Visiting Fellow	LDB, NIDR
	Yamada SS	Special Volunteer	LDB, NIDR

COOPERATING UNITS *(if any)*

University of Texas, Dallas (Grinnell F); CNRS, France (Thiery JP); Kyoto University, Japan (Takeichi M); University of Turku, Finland (Larjava H); University of Helsinki, Finland (Thesleff I).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	2.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Integrins and other cell surface receptors for extracellular matrix proteins such as fibronectin and vitronectin play roles in embryonic development and wound healing. Our studies suggest important roles for integrins in early development, such as during tooth morphogenesis and neural crest cell migration. Defects in integrin-related functions may contribute to a variety of human congenital defects involving mistakes in morphogenesis, as well as contributing to poor wound healing. Recent studies in progress have identified unexpectedly rapid and precise changes in expression of mRNA for the β5 integrin during mouse molar development. Large, stage-specific changes in β5 mRNA levels were detected, with alternation of expression between epithelium and mesenchyme within a single day. Such major regulatory switching is unprecedented for integrins, and it suggests interesting regulatory roles during tooth development. Adult keratinocytes are activated for adhesion and migration during the process of wound repair. Collaborative studies show that novel glycosylation changes in integrins accompany this process, which can be mediated by serum factors. Inhibition of β1 integrin function in keratinocytes leads to disruption of actin cytoskeletal organization, even though cadherin cell adhesion molecules are apparently unaffected in distribution. This result provides an approach to understanding the functional roles of the actin scaffolding in cells by selective perturbation. In an ongoing reagent development program, we have developed novel recombinant DNA and immunological probes against various integrin subunits and their ligands to be able to test their roles in early development using animal models, e.g. in the formation of craniofacial structures. New mouse cDNA probes are now available for αv, β3, β5, β6, and other integrins, as well as for murine fibronectin and vitronectin. New antibody probes for α3 integrin and vitronectin are also being characterized. Other studies will characterize the mutual cross-regulation of function of integrin and cadherin adhesion systems during morphogenesis and other developmental events.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00525-03 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms and Regulation of Cell Adhesion, Migration, and Morphogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Yamada KM	Chief	LDB, NIDR
Others:	Aota S	Visiting Associate	LDB, NIDR
	Thomas LA	Biologist	LDB, NIDR
	Lee C-C	Visiting Fellow	LDB, NIDR
	Tran MN	Biologist	LDB, NIDR
	Savagner P	Special Volunteer	LDB, NIDR
	Yamada SS	Special Volunteer	LDB, NIDR

COOPERATING UNITS *(if any)*

CBER, FDA (Komoriya A, Shinagawa S); Dept. Anatomy, Univ. Pennsylvania (Lash J).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.27	3.6	1.67

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cell adhesion, migration, and morphogenesis are crucial events in craniofacial development, and errors in them can produce congenital anomalies. Specific structural molecules that mediate these processes and the mechanisms that regulate them are being identified and characterized in detail. Fibronectin and related molecules appear to be crucial for normal morphogenesis, e.g. for neural crest cell migration to form craniofacial structures. The regions of fibronectin that are essential for cell adhesion are being characterized in detail by site-directed mutagenesis and sequence homology scanning approaches. A 5 amino acid sequence in the 9th type III repeating unit has been tentatively identified as essential for cell adhesion mediated by the fibronectin receptor. Precise mapping of this site should facilitate the rational design of novel, specific bioadhesives and competitive inhibitors. Cell migratory interactions with extracellular molecules can be regulated by a novel regulatory phenomenon we have discovered termed "contact stimulation of migration." Melanocytes and neural crest cells display 10-200 fold stimulation of migration rates after such cell-cell contact. The molecular mechanisms of this process are being pursued. Another mechanism for regulating cell migration involves cytokines such as scatter factor/hepatocyte growth factor and its c-met proto-oncogene receptor. We have sequenced mouse scatter factor, which has 90% amino acid sequence identity with its human homologue. The mRNA for this molecule can be detected in substantial quantities in 9-10 day mouse embryos, suggesting that it may play roles in developmental regulation of cell type and migration. The corresponding mouse c-met receptor cDNA has also been cloned. We have discovered a novel spliced version of this receptor that may have distinct regulatory properties, a possibility that is being tested using an expression system to compare standard and alternatively spliced products. These studies should provide a molecular understanding of how the complex but important processes of cell migration during embryogenesis are regulated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00559-02 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological Activities of HIV Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kleinman HK	Section Chief	LDB, NIDR
Others:	Yamada KM	Chief	LDB, NIDR
	Weeks BS	Biologist	LDB, NIDR
	Holloway E	Biol. Lab Tech.	LDB, NIDR
	Johnson BA	IRTA Fellow	LDB, NIDR

COOPERATING UNITS *(if any)*

St. Louis University, (Green M, Desai K); NCI, NINCDS (Lieberman D).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.03	1.86	1.17

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In patients with HIV infection (AIDS), there is an unexplained dementia. Active virus is not observed in brain cells so it is proposed that a soluble factor released from the virus may be affecting the patients. We previously found that the HIV viral protein TAT (which is a transactivator of the HIV-LTR promoter) promotes neural cell adhesion in vitro and blocks laminin-mediated process outgrowth. Using recombinant TAT, synthetic TAT (it is 86 amino acids long) and smaller synthetic peptides duplicating sequences in TAT, residues 49-57 were found to be responsible for the biological activity. Using peptide affinity chromatography and coimmuno-precipitation of labeled cell membranes, a 90 kd TAT receptor on neuronal cells was identified. Direct injection of TAT into the brains of rats caused impaired motor function and destruction of large amounts of brain tissue often resulting in death. The smaller peptides were without activity. Studies are focussing on characterizing the receptor and defining the mechanisms of TAT toxicity on neural cells. These data demonstrate that TAT has a strong effect on neural cells and suggest a possible mechanism to explain the neurologic changes and dementia observed in AIDS patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00560-02 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Functional Analysis of Integrin Cytoplasmic Domains

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	LaFlamme SE	Senior Staff Fellow	LDB, NIDR
Others:	Yamada KM	Chief	LDB, NIDR
	Akiyama SK	Research Chemist	LDB, NIDR
	Miyamoto S	Special Volunteer	LDB, NIDR
	Yamada SS	Special Volunteer	LDB, NIDR

COOPERATING UNITS *(if any)*

Oncology, John Hopkins Hospital, Baltimore, MD, (Tucker R, Wilhide C); Dept. Immunopathology, Scripps Research Institute, La Jolla, CA, (Ginsberg M).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.23	1.43	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Integrins are the major class of receptors by which cells interact with the extracellular matrix, promoting cell adhesion and cell migration. Signaling from extracellular matrix molecules to the interior of cells can occur via integrin receptors. These signaling events are thought to play roles in processes important for embryonic development such as tissue organization, cell migration, and protease secretion. Integrins generally contain two relatively short cytoplasmic domains. The roles of these domains in controlling the location of receptors, signaling, and regulating cell behavior is being explored using molecular biology and biochemical methods. Functions of isolated domains are being tested using chimeric receptors containing a reporter domain consisting of a subunit of the interleukin-2 receptor and various integrin cytoplasmic tails. The β1, β3, and to some extent β5 integrin cytoplasmic domains were found to contain sufficient information for the targeting of receptors to adhesion sites of cells, whereas the α5 and alternatively spliced version of the β3 integrin do not. Further studies are characterizing the roles of integrin cytoplasmic domains in regulating messenger systems involving tyrosine phosphorylation, calcium concentration, and pH. Overexpression of certain cytoplasmic domain chimeras was able to produce a dominant negative phenotype. The β1 and β3 cytoplasmic domains can inhibit cell spreading and localization of endogenous fibronectin receptors to extracellular matrix contacts with fibronectin fibrils; the alternatively spliced β3 and β5 cytoplasmic domain chimeras could not. These studies demonstrate the central role of certain specific integrin cytoplasmic domains in cellular functions. They should help provide an in-depth understanding of how cells communicate with their extracellular environment in normal and abnormal embryonic development, where such signaling is essential for coordinating the complex rearrangements and final organization of oral, facial, and other developing tissues. These interactions are also likely to be important for adult tissue repair.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00563-02 LDB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Cell-Substrate Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Akiyama SK	Senior Staff Fellow	LDB, NIDR
Others:	Yamada KM	Chief	LDB, NIDR
	Tran MN	Biologist	LDB, NIDR
	Aota S	Visiting Associate	LDB, NIDR
	LaFlamme S	Senior Staff Fellow	LDB, NIDR
	Torchia D	Chief, PBS	BRB, NIDR
	Copie V	Guest Researcher	BRB, NIDR

COOPERATING UNITS *(if any)*

Dept of Periodontics, Univ of Turku School of Dentistry, Finland (Larjava H); Dept of Cell Biol, Weizmann Inst of Science, Israel (Lider O); BRB, NIH (Copie V, Torchia D).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The adhesive protein fibronectin and its integrin receptors play important roles in embryonic development, wound healing, and the progression of diseases such as cancer. Techniques involving monoclonal antibodies, molecular and cell biology, and physical biochemistry are being used to elucidate molecular mechanisms of fibronectin-receptor interactions with the goal of understanding the roles of these glycoproteins in complex biological processes in order to develop novel bioadhesive substrates and to provide the bases for rational medical intervention in diseases involving abnormal cellular adhesion and migration. Either cell adhesion to integrin-binding adhesive proteins or integrin clustering results in signal transduction in the form of tyrosine phosphorylation of an intracellular protein called the focal adhesion kinase. Simple occupancy of fibronectin-binding integrins with soluble ligand fragments is insufficient to stimulate tyrosine phosphorylation. Likewise, tyrosine phosphorylation occurs more rapidly than does formation of focal adhesions, suggesting that these structures are not required for signalling to occur. However, the abilities of different integrin β subunit intracellular domains to mediate tyrosine phosphorylation parallels their abilities to spontaneously cluster at focal adhesion sites, suggesting a connection between these two processes. Another form of integrin-mediated transmembrane signalling has been investigated in human gingival keratinocytes. Antibodies and Fab fragments that bind to the α3β1 integrin stimulate the expression of the 92 kDa type IV collagenase independent of ligand binding by integrins as well as the adhesive substrate being used by the cells. However, expression of the 92 kDa type IV collagenase can also be stimulated by TGF-β1 and TPA. The biological activities and structure of the bacterially-expressed 20 kDa fibronectin cell-adhesive region spanning the ninth and tenth type III repeats has been further characterized. When immobilized using non-inhibitory monoclonal antibodies, this fragment promotes cell adhesion and migration with a similar activity as intact fibronectin, suggesting that it might have potential value as a bioadhesive and in promoting wound healing. Immobilized fibronectin has been found to bind tumor necrosis factor-α (TNF-α) via its amino-terminal domain. Fibronectin-bound TNF-α also appears to enhance integrin-mediated cell adhesion to fibronectin. These results suggest that fibronectin or fibronectin fragments may play a role in the modulation of inflammatory responses involving TNF-α.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00034-25 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Histamine Release

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.; Chief, Receptors and Signal Transduction Section, LI, NIDR
Hook, William A.; Research Microbiologist, LI NIDR
Hamawy, Majed M.; Staff Fellow, LI NIDR
Mergenhagen, Stephan E.; Chief, Laboratory of Immunology, NIDR
Berenstein, Elsa H.; Microbiologist - Minoguchi, Kenji; Visiting Fellow, LI NIDR
Swieter, Mark; Senior Staff Fellow, - Kihara, Hidetoshi; Visiting Fellow, LI NIDR
Benhamou, Marc; Visiting Associate, LI NIDR
Bader, Greta; Biologist, LI NIDR; Tomlinson, Nicola; Visiting Fellow, LI NIDR

COOPERATING UNITS *(if any)*

Dr. Marvin Karten NICHD ODCPR, NIH

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
9.55	8.55	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Histamine release from mast cells and blood basophils is being studied as one of the immunological mechanisms involved in inflamation. It is also a model for cell secretion. Among the histamine releasing agents employed are IgE antibody, other secretagogues, LHRH peptides, and the calcium ionophore A23187. Cultured rat b asophilic leukemia cells are used as a model for the studies of the IgE receptor and of biochemical changes during cell activation. Large numbers of cells can be obtained for biochemical studies and biochemical variants have been selected which are defective at different sites in the pathway of cell activation and secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00046-22 LI

PERIOD COVERED

October 01, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Normal and Pathologic Mechanisms of Inflammation and Repair.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Sharon M. Wahl	Chief, CIS	LI, NIDR
	Mary Slater-Venkata	Biologist	LI, NIDR
	Lauren Flores	PRAT Fellow	LI, NIDR
	Christopher Romann	Stay-In-School	LI, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	.7	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transforming growth factor β (TGF-β), secreted within an inflammatory site or injected locally, induces leukocyte margination, chemotaxis, and accumulation. In addition to its potent direct chemotactic activity, TGF-β promotes this leukocyte response by influencing cell surface integrin expression. At picomolar concentrations, TGF-β increases steady-state mRNA levels for both the α_5, α_3, and β_1 molecules on the cell surface. Functionally, TGF-β promotes, in a dose- and time-dependent fashion, monocyte adhesion to type IV collagen, laminin, and fibronectin. TGF-β also triggers transcriptional and posttranscriptional regulation of type IV collagenase. Thus, TGF-β may play a pivotal role in the early phases of inflammation and repair through its ability to mediate monocyte adhesion, chemotaxis, and enzymatic digestion of extracellular matrix, whereas in chronic lesions, excess TGF-β may contribute to persistent leukocyte accumulation. Therefore, in exploring potential antagonists of TGF-β, we have identified the Th2-derived cytokine, IL-4, as an endogenous inhibitor of TGF-β-stimulated monocyte functions including adhesion and collagenase production. Interestingly, TGF-β-stimulated monocytes expressed elevated levels of IL-4 receptor mRNA and protein, augmenting their susceptibility to the anti-inflammatory effects of IL-4. In additional studies, IL-4 was shown to suppress both TGF-β and IL-1β gene expression induced by TGF-β. Suppression of IL-1β by IL-4 occurred subsequent to TGF-β interaction with its receptor and signalling, and was regulated at the transcriptional level. Coincident with the suppression of IL-1β, IL-4 augmented TGF-β-induced IL-1 receptor antagonist (IL-1ra) production, expanding its anti-inflammatory potential. Thus, these data indicate that IL-4 antagonizes the inflammatory actions of TGF-β on immature monocytes, but works together with TGF-β to mediate immune suppression by deactivating stimulated monocyte/macrophages and by inducing IL-1ra.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00199-17 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Studies of Secretory Cell Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Oliver, Constance; Guest Researcher Biologist, LI NIDR
Siraganian, Reuben P.; Chief, Receptors and Signal Transduction Section, LI NIDR
Waters, Judith F.; Biologist, LI NIDR
Weedon, Lynda L.; Biologist, LI NIDR
Swaim, William D.; IRTA Fellow, LI NIDR

COOPERATING UNITS *(if any)*

Dr. A. Robbins, LBM NIDDK

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.25	2.25	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Secretory and endocytic processes in several cell types are currently under investigation. The rat basophilic leukemia cell line (RBL-2H3) and other cultured cells are being used to study various aspects of endocytic and secretory processes. Emphasis is placed on morphological, cytochemical and biochemical characterization of these processes in the cultured cells. Events involved in receptor activation, signal transduction and endocytic mechanisms are under investigation. The lysosomal system and its role in endocytic and secretory pathways is also under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00290-14 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Production of Hybridomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Siraganian, Reuben P.; Chief, Receptors and Signal Transduction Section, LI NIDR
Berenstein, Elsa H.; Microbiologist, LI NIDR
Fischler, Cynthia; Biological Lab Technician, LI NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	1.25	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hybridomas are being produced which secrete monoclonal antibodies of defined antigen specificity. Hybridomas have been produced against the Fcε receptor of mast cells and to human IgE. These monoclonal antibodies are being used for biochemical and biological studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00424-08 LI

PERIOD COVERED

October 1, 1992 - September 30,1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Monocyte Phenotype and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

McCartney-Francis, Nancy	Senior Expert	LI, NIDR
Wahl, Sharon	Chief, CIS	LI, NIDR
Wahl, Larry	Senior Investigator	LI, NIDR
Mizel, Diane	Chemist	LI, NIDR
Shepyheard, Sharnn	NRSA	LI, NIDR

COOPERATING UNITS *(if any)*

L.E. Algina, M.D., Dept. Surgery, Rhode Island Hospital, Providence, RI; Q.-w. Xie, Ph.D., C.F. Nathan, M.D., Dept. Medicine, Cornell University Medical College, New York, NY; C. Manthey, Ph.D., Dept. Microbiol., Uniformed Services University HEalth

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.05	2.05	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this research program is to define the phenotypic and functional properties of monocytes and the molecular mechanisms that regulate the inflammatory process. One effector molecule that has been implicated as a mediator of immune and inflammatory responses is nitric oxide (NO), a toxic radical gas produced during the metabolisn of L-arginine by NO synthase (NOS). Previous studies have identified increased levels of nitric oxide in synvovial fluids from rheumatoid arthritis patients. These studies provided evidence that nitrogen intermediates might contribute to pathologic sequelae in chronically inflamed tissue. A single injection of streptococcal cell walls (SCW) induces the accumulation of inflammatory cells within the synovial tissue and a cell-mediated immune response that leads to destructive lesions. NO production is elevated in the inflamed joints of SCW-treated rats. To address the possible therapeutic intervention of the nitric oxide pathway, arthritic rats were treated with daily injections of N^G-monomethyl-L-arginine, an analog of arginine and inhibitor of NOS. NMMA profoundly reduced the synovial inflammation and tissue damage, implicating the NO pathway in the pathogenesis of inflammatory arthritis and demonstrating the ability of a NOS inhibitor to modulate the disease. Elevated nitrite levels have also been demonstrated in other inflammatory and infectious diseases such as periodontal disease and AIDS, suggesting that NOS inhibitors may be useful in the diagnosis and treatment of these and other inflammatory and immune mediated disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00441-07 LI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Experimentally Induced Immune Responses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Tomozumi Imanichi	Fogarty Fellow	LI, NIDR
	Sharon M. Wahl	Chief, CIS	LI, NIDR
	Nancy Francis	Special Expert	LI, NIDR
	Keith Hines	PRAT Fellow	LI, NIDR
	John Zagorski	Staff Fellow	LI, NIDR
	Marielle Christ	Visiting Fellow	LI, NIDR

COOPERATING UNITS *(if any)*

M. Bienkowski, A. Berger, Hoffman-LaRoche; C. Manthey, USUHS; J. Dasch, Celtrix Laboratories; B. Sartor, UNC-Chapel Hill

LAB/BRANCH

Laboratory of Immunolofy

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Challenge of Lewis (LEW/N) rats with SCW induces acute and chronic inflammation in peripheral joints, liver, and spleen. In contrast, the inbred MHC compatible Fischer strain (F344/N) does not develop chronic inflammatory and autoimmune-like disease. To identify potential differences at the genetic level which might contribute to the phenotypic differences between the LEW/N and F344/N strains, animals of both strains were injected with SCW, and a subtracted cDNA library (LEW/N - F344/N) was generated from peritoneal exudate cells. Subsequent analysis of differentially expressed genes revealed clones which exhibit homology to human migration inhibitory factor-related proteins (MRP). These calcium-binding proteins form complexes, are expressed by circulating monocytes and neutrophils and are expressed during the development of arthritis. The correlation of MRP8 and MRP14 expression with genetic susceptibility to the development of chronic inflammation in response to bacterial cell wall antigens provides new opportunities for defining molecular mechanisms responsible for potentially pathologic inflammatory diseases. In additional studies, peptides synthesized from fibronectin which inhibited leukocyte adhesion in vitro were administered to arthritic animals either as free peptides or coupled to a carrier molecule. Peptides containing either the RGD or CS-1 cell-binding domains were inhibitory to chronic synovial pathology, as were three peptides synthesized from the carboxyl terminal 33 kD heparin-binding domain. Since transforming growth factor β (TGF-β) induces leukocytes adhesion, antagonism of TGF-β with a neutralizing antibody also blocked inflammatory cell accumulation and tissue pathology in this model. These data implicate TGF-β as a profound antagonist not only in the early events responsible for synovial inflammation, but also in the chronicity of SCW-fragment-induced inflammation culminating in destructive pathology. Interrupting the cycle of leukocyte recruitment and activation with TGF-β antagonists or synthetic peptides may provide mechanisms for resolution of chronic destructive lesions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00456-06 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Signal Transduction in the monocyte/macrophage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Larry M. Wahl	Research Biologist	LI, NIDR
Prema Mertz	Staff Fellow	LI, NIDR
Marta Corcoran	Chemist	LI, NIDR
Susan Hopkinson	Chemist	LI, NIDR

COOPERATING UNITS *(if any)*

D.S. Finbloom, FDA; I. Katona, USUHS; W. Stetler-Stevenson, NCI; I. Horak, NCI

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.05	1.45	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focusses on the biochemical events involved in the PGE2-cAMP dependent signal transduction in the monocyte that leads to the production of metalloproteinases. This last year the emphasis of this research has been on the mechanisms by which cytokines regulate the events involved in this pathway. Of particular interest is the effect of cytokines on the recently described inducible form of prostaglandin synthase known as prostaglandin H synthase-2 (PGHS-2). Preincubation of monocytes with interleukin-4 (IL-4) caused a significant suppression of Con A induced PGHS-2 as demonstrated at the mRNA and membrane protein level. This appears to be the primary mechanism by which IL-4 inhibits the production of PGE2 and the subsequent production of metalloproteinases since phospholipase activity, as assessed by HPLC analysis, appears to be relatively unaffected by IL-4. Interleukin-10 is another cytokine, that as a result of its suppression of mRNA and membrane protein levels of PGHS-2, was found to inhibit matrix metalloproteinase synthesis by monocytes. In contrast to IL-4 and IL-10, transforming growth factor beta (TGFβ) was shown to enhance the induction of PGHS-2 mRNA and protein by Con A. The ability of TGFβ to enhance PGHS-2 was also reflected in increased production of prostaglandins, including PGE2. While TGFβ enhanced monocyte eicosanoid synthesis, matrix metalloproteinase was not consistently increased by TGFβ, indicating that this cytokine may be affecting additional signalling events in this pathway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00513-04 LI

PERIOD COVERED

October 01, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Monocytes in AIDS and as Targets for Antiviral Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lauren Flores	PRAT Fellow	LI, NIDR
	Phillip Smith	Senior Investigator	LI, NIDR
	Tessie McNeely	Sr. Staff Fellow (CRADA)-No Staff Years	LI, NIDR
	Marian Dealy	Biologist, (CRADA) - No Staff Years	LI, NIDR
	Sharon Wahl	Chief, CIS	LI, NIDR
	Nandita Chopra	Biologist	LI, NIDR

COOPERATING UNITS *(if any)*

S. Eisenberg, and R. Thompson, Synergen, Boulder, CO.; Jan Orenstein, GWU; Randall Wagner, GWU; J.B. McCarthy, Univ. Minnesota

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.45	1.45	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monocytes and macrophages express CD4 on their surface and are targets for HIV. Infection of phagocytic cells is not cytotoxic, and these chronically infected cells may serve as a reservoir for HIV. Continued characterization of the functional abnormalities of this population is essential to understanding the immunosuppressed state of AIDS patients. Recent studies have demonstrated that HIV infection induces production of a cytokine unique to monocytes and macrophages: the interleukin-1 receptor antagonist (IL-1ra). This cytokine is secreted by activated phagocytes, binds to the IL-1 receptor, but does not transduce a signal. IL-1ra is unique in that it is the only known endogenous receptor antagonist. By binding to the IL-1 receptor, IL-1ra attenuates the pro-inflammatory actions of IL-1. Monocytes produce both IL-1 and IL-1ra, and the relationship between these two cytokines is crucial to maintaining the proper balance between immune cell recruitment/activation and immunosuppression and tissue repair. In addition to influencing immune status, infected monocytes serve as a reservoir for HIV-1 favoring selective therapeutic targeting of this population. Interaction of HIV-1 with cell surface CD4 transduces a signal inducing secretion of cytokines, and also *de novo* expression of activation antigens. For example, monocytes from HIV^+ individuals maybe $CD16^+$ and $IL\text{-}2R^+$ in contrast to monocytes from control subjects. The emergence of IL-2R on infected cells provides the basis for targeted toxins. Current studies are focusing on other alterations in the expression of antigens and surface receptors as selective toxin targets for therapeutic intervention.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00561-02 LI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Taste and Smell

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ryba, Nicholas; Visiting Associate; LI NIDR
Hall, Matthew; Visiting Fellow; LI NIDR
Hirano, Fuki; Guest Researcher; LI NIDR
Hoon, Mark; Visiting Fellow; LI NIDR
Siraganian, Reuben; Chief, Receptors and Signal Transduction, LI NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

'NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.25	4.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular mechanisms of signal reception and transduction in taste and smell are being studied. Synthetic genes for the sweet-tasting proteins are being expressed for production of a high affinity ligands for taste receptor identification and characterization. A soluble binding protein and a G-protein that appear to function in taste discrimination and signal transduction respectively have been expressed and are being studied in situ. A novel G-protein γ-subunit from the basal (stem) cells and developing neurons of the olfactory and vomeronasal epithelia has been cloned and functional studies are in progress. Several putative olfactory receptors are also being expressed for functional characterization; antibodies are being raised to study these native and recombinant receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00043-23 LME

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological and Genetic Studies of Oral and Other Microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Donkersloot, Jacob A.	Research Microbiologist	LME, NIDR
Harr, Robert J.	Biolaboratory Technician	LME, NIDR
Thompson, John	Visiting Scientist	LME, NIDR
Robrish, Stanley A.	Research Microbiologist	LME, NIDR
Pikis, Andreas	Staff Fellow	LME, NIDR

COOPERATING UNITS *(if any)*

David W. Rice, Dept. of Molecular Biology and Biotechnology, University of Sheffield, United Kingdom

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.15	1.35	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It has recently been found that the Gram-negative anaerobe *Fusobacterium mortiferum* FM1 (ATCC 25557) metabolizes maltose via an inducible phosphoenolpyruvate:maltose phosphotransferase system (PTS). The maltose 6-phosphate formed from this group translocation is subsequently converted to glucose 6-phosphate and glucose by the enzyme maltose 6-phosphate hydrolase (S. Robrish and J. Thompson, manuscript in preparation). To gain insight into the substrate specificity and regulation of this maltose system, a maltose-negative mutant was isolated. Whereas this mutant (FM12) grew on sucrose (another PTS sugar), it did not grow on maltose, isomaltose, and palatinose. In contrast to the parent, FM12 cells also did not produce p-nitrophenol from p-nitrophenyl α-glucoside. This latter property was used to demonstrate a requirement for phosphoenolpyruvate and anaerobiosis for the concerted EII^{mal} and maltose 6-phosphate hydrolase mediated conversion of p-nitrophenyl α-glucoside to p-nitrophenol by permeabilized FM1 cells. Isomaltose was fermented more rapidly than maltose by the parent strain, which suggested that this sugar could be a precursor of the intracellular inducer. Indeed, as little as 0.1 mM isomaltose stimulated growth on trehalose, which by itself does not support growth of non-induced FM1 cells. A constitutive mutant was isolated which grew (without a lag) on maltitol, α-methyl glucoside, trehalose, turanose, and palatinose. These α-glucosides are also catabolized by maltose-induced FM1 cells, but do not support growth of non-induced cells. Thus, it appears that the maltose system of *F. mortiferum* has an unusually broad substrate specificity for α-glucosides, but that the induction process is much more specific.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00254-16 LME

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microbial Antigens Associated with Specific Adherence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cisar, John O.	Research Microbiologist	LME, NIDR
Hsu, S. Dana	Microbiologist	LME, NIDR
Takahashi, Yukihiro	Visiting Fellow	LME, NIDR
Sandberg, Ann L.	Chief, Microbial Receptors & Pathogenesis Sect.	LME, NIDR

COOPERATING UNITS *(if any)*

University of Florida; University of Maryland; Royal Dental College Aarhus, Denmark, Georgetown University

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbial Receptors and Pathogenesis Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Structural studies showed that the *Streptococcus gordonii* 38 receptor polysaccharide was composed of a repeating heptasaccharide linked end to end by phosphodiester bonds. The heptasaccharide repeating units of strain 38 and *S. mitis* J22 differed only at their reducing ends with GalNAcβ1→3Gal in the former and Galβ1→3GalNAc in the latter while a rhamnose branch near the nonreducing end of the strain 38 heptasaccharide distinguished this structure from the linear hexasaccharide of *S. oralis* 34. Certain strains of *S. sanguis* and *S. gordonii* that express GalNAc-sensitive lectins coaggregated with strains 38 and 34 which have GalNAcβ(1→3)Gal-containing receptor polysacchides but not with *S. mitis* J22 which has a Galβ1→3GalNAc containing receptor polysaccharide. Presumably, the complimentarity of these lectins involves the side of GalNAcβ1→3Gal that includes the acetamido group of GalNAcβ. In contrast, actinomyces interact with both types of receptor polysaccharides which suggests lectin complimentarity for a side of GalNAcβ1→3Gal that is shared with Galβ1→3GalNAc. The antigenic properties of these polysaccharides appear to depend on structural features distinct from those detected by lectin binding. For example, the presence of the common rhamnose branch accounts for the strong cross reactivity between the strain 38 and J22 polysaccharides. A structural understanding of these cross reactions provides a rational basis for the preparation of specific immunological reagents.

Studies were also initiated to characterize the adhesive properties of viridans streptococci that colonize the human oral cavity shortly following birth and also following the eruption of teeth. Whereas isolates obtained during the first two months were relatively nonadherent, isolates obtained after tooth eruption exhibited an array of adhesive properties similar to those noted in studies of adult isolates. The results clearly implicate specific bacterial adhesive properties as important determinants of in vivo colonization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00273-15 LME

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell-Cell Interactions Between Oral Actinomyces And Other Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Kolenbrander, Paul	Research Microbiologist	LME, NIDR
Ms. Andersen, Roxanna	Microbiologist	LME, NIDR
Dr. Clemans, Daniel	Guest Researcher	LME, NIDR
Dr. Ganeshkumar, Nadarajah	Visiting Associate	LME, NIDR
Dr. Klier, Christiane	Visiting Fellow	LME, NIDR
Dr. London, Jack	Research Microbiologist	LME, NIDR
Ms. Roble, Arlene	Summer IRTA	LME, NIDR

COOPERATING UNITS *(if any)*

Dr. L.V.H. Moore, VPI and SU, Blacksburg, VA; Dr. B.C. McBride, University of British Columbia, Vancouver, Canada; Dr. E. Weiss, Tel Aviv University, Tel Aviv,

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.67	3.42	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of our research program is to understand the role of coaggregation in bacterial accretion of early colonizing bacteria on a clean tooth surface. The primary colonizers include actinomyces and streptococci. Antiserum against the 34.8-kDa surface-adhesin, ScaA, from *Streptococcus gordonii* PK488 cross reacts with a similar size protein from all 14 streptococcal strains so far tested that coaggregate with *Actinomyces naeslundii* PK606. The sequence of the 6.125 kb clone that contains *scaA* revealed six open reading frames. The adhesin gene is located adjacent to two genes encoding an ATP-binding protein and a hydrophobic membrane protein. These three genes are homologous to a cassette of genes encoding binding-protein dependent transport systems in several other bacteria. ScaA is a lipoprotein which probably is anchored in the cell membrane while its adhesin binding site is exposed to the environment. Two other oral streptococci also have the same genetic organization of these genes, suggesting that the adhesin is a critical function for colonization of oral streptococci.

A 100-kDa protein thought to mediate intrageneric coaggregation among streptococci is absent in several spontaneous mutants and one transposon-inactivated mutant of *Streptococcus gordonii* DL1. Two potential coaggregation-mediating adhesins from *Actinomyces* serovar WVA963 strain PK1259 have been identified. Binding of oral actinomyces to defined glycolipid molecules appears to follow the sema specificity exhibited by the actinomyces coaggregations with oral streptococci. The long range goal of these studies, collectively, is to elucidate the molecular mechanisms responsible for bacterial colonization in the human oral ecosystem.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00341-12-LME

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of sugar transport and metabolism in lactic acid and oral bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thompson, John	Visiting Scientist	LME, NIDR
Donkersloot, Jacob A.	Research Microbiologist	LME, NIDR
Robrish, Stanley A.	Research Microbiologist	LME, NIDR
Gentry-Weeks, Claudia R.	Sr. Staff Fellow	LME, NIDR

COOPERATING UNITS *(if any)*

Miller, Stephen P.F.	Staff Fellow	DMNB, NINCDS
Fales, Henry	Lab Chief,	LBC, NHLBI
Davidson, Barrie E.	Professor	University of Melbourne

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal(s) of this program are to understand the molecular basis, and the roles of microorganisms in the etiology and pathogenesis of oral diseases. The products of microbial metabolism, including organic acids and sulfur-containing compounds, are either causative or contributory agents to the development of dental caries, gingivitis and periodontitis. Consequently, our research efforts are concerned with the elucidation of the biochemical, enzymatic, and genetic factors, responsible for the fermentation of sugars and amino acids by oral microorganisms. Major accomplishments during the past year include:

1. The identification of a novel phosphoenolpyruvate-dependent maltose: phosphotransferase system in *Fusobacterium mortiferum*.
2. The purification to homogeneity of two enzymes required for the metabolism of sugars by lactic acid bacteria. Fructokinase II catalyzes the ATP-dependent phosphorylation of fructose, whereas triosephosphate isomerase is a key enzyme for the fermentation of sugars via the Embden-Meyerhof-Parnas (glycolytic) pathway.
3. Site-directed mutagenesis has facilitated identification of substrate binding and catalytically functional residues in N5-(carboxyethyl)ornithine synthase.
4. Beta-Cystathionase has been purified, cloned and sequenced from *Bordetella avium*. The biochemical basis for the toxicity of this (pyridoxal-phosphate containing) protein toward osteogenic cells has been established. Site-directed mutagenesis experiments have provided evidence for participation of the lysyl residue K214 in co-factor binding, and for the role(s) of cysteine residues in the catalytic process.
5. Antibodies prepared against Beta-cystathionase from *B. avium* have been used to screen for the same (or, related) enzyme(s) in extracts of oral pathogens including Fusobacteria and Bacteroides species.

Results from this research program have been published in peer-reviewed Journals, and have also been discussed in a textbook chapter dealing with the regulation of sugar transport and metabolism by lactic acid bacteria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00382-10 LME

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth and Metabolism of Oral Microorganisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Robrish, Stanley A.	Research Microbiologist	LME, NIDR
	Thompson, John	Visiting Scientist	LME, NIDR
	Gomez, Irma M.	Microbiologist	LME, NIDR
	Gentry-Weeks, Claudia	Sr. Staff Fellow	LME, NIDR
	Donkersloot, Jacob	Research Microbiologist	LME, NIDR

COOPERATING UNITS *(if any)*

Fales, Henry	Laboratory Chief	LBC, NHLBI

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Maltose grown cells of *Fusobacterium mortiferum* ATCC 25557 were able to transport maltose, in contrast to cells from cultures grown on other energy sources. Maltose grown cells were also able to use both maltose and sucrose, as well as a variety of alpha linked glucosyl disaccharides, anaerobically from a buffered cell suspension. Permeabalized cells and sonic extracts of maltose grown *F. mortiferum* phosphorylate maltose with phosphoenolpyruvate [PEP] exclusively as the phosphate donor. Permeabalized cells from cultures grown on serine, glucose or sucrose were unable to phosphorylate maltose with either adenosine triphosphate [ATP] or PEP as a phosphate donor. PEP stimulated glucose phosphorylation was found in cells grown on all four energy sources and was strongest in maltose grown cells. When a sonic extract of *F. mortiferum* was resolved into membrane and cytosolic fractions neither component could affect PEP dependent maltose phosphorylation alone but maltose PTS activity was restored by addition of both components. The cystosolic fraction from either glucose or maltose grown cells complimented maltose membranes for maltose PTS activity. A glucose PTS was demonstrated in maltose grown cells.

Aerobic conditions allowed PEP dependent maltose phosphorylation but prevented further disaccharide dissimilation suggesting aerobic sensitivity of a putative maltose-6-phosphate hydrolase. Maltose-6-phosphate hydrolase activity could not be demonstrated using cell extracts although evidence with intact anaerobic cells showed conclusively that the activity was present. Maltose-6-phosphate was prepared in milligram amounts using permeabalized maltose grown *F. mortiferum*, maltose and PEP as described herein. The structure of the compound was proved by chromatographic identification of products following enzymatic and chemical treatments. The proposed structure was confirmed using mass spectral data and nuclear magnetic resonance spectroscopy.

Cystathionase [cystine lyase] was detected by activity stain in a gel following separation of sonic extracts of a variety of fusobacteria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00454-07 LME

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Surface Molecules in Colonization and Biological Mimicry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	London, Jack P.	Research Microbiologist	LME, NIDR
	Allen, Janet	Microbiologist	LME, NIDR
	Bouma, Carolyn	Staff Fellow	LME, NIDR
	Kolenbrander, Paul E.	Microbiologist	LME, NIDR
	Lunsford, R.D.	Staff Fellow	LME, NIDR
	Riley, Chiara	Staff Fellow	LME, NIDR
	Cavedon, Kathrine	IRTA	LME, NIDR

COOPERATING UNITS *(if any)*

Dr. A. Hand, University of Connecticut, Dr. J. Manch-Citron, University of Missouri and Dr. I. Weiss, Tel Aviv University, Tel-Aviv Israel

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.39	2.39	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study seeks to define, at the genetic and structural level, the *Prevotella loescheii* fimbrial-associated adhesins and the fimbriae bearing them. After cloning and sequencing the galactoside-specific fimbrial-associated adhesin gene, the interrupted translation region within the mature protein beginning at the codon for amino acid 29 (actually an ochre terminator) was characterized. Existence of a stretch of 138 nucleotides containing the two ochre terminators, a polyA slip region, stem loop and pseudoknot was demonstrated in genomic DNA and adhesin mRNA establishing that ribosomal translation requires a bypass or "hop" of at least 29 nucleotides located between two ochre codons. The entire region was replicated by PCR and fused in frame into the 5′ terminus of *lacZ* to demonstrate that *E. coli* is capable of reading through the bypass region efficiently; expression of the gene occurred at levels varying between 4 and 25% of the control.

A DNA probe based on the sequence of an internal 10 amino acid peptide isolated from a digest of pure *P. loescheii* actinomyces-specific adhesin reacted with restricted genomic DNA from this oral bacterium. The probe was subsequently used to screen a GT-11 lambda phage genomic *P. loescheii* library. Currently, DNA containing the phage-positive insert is being isolated for cloning.

Similarly, the N-terminal sequence of *P. loescheii* fimbriae was determined from a purified preparation of the structures. A radioactive oligonucleotide probe was prepared following reverse translation and was found to react with digested genomic DNA. The above mentioned *P. loescheii* lambda GT-11 library is currently being screened with the oligonucleotide.

The ribitol-5-P dehydrogenase gene found in the ribitol operon has been cloned and is roughly 50% sequenced. The ribitol PTS transport is currently being sought in the flanking regions of the dehydrogenase gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00512-04 LME

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic analysis of *Bordetella* and *Fusobacterium* pathogenicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Claudia R. Gentry-Weeks	Senior Staff Fellow	LME, NIDR
John Thompson	Visiting Scientist	LME, NIDR
Stanley Robrish	Research Microbiologist	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Instiute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bordetella avium 197 produces a dimeric, 42.6 kDA protein ('osteotoxin') which is toxic for MC3Te-E1 osteogenic cells. Genetic analyses and enzymatic characterization of the purified protein established that the osteotoxic protein was beta-cystathionase (encoded by metC), an enzyme in the biosynthetic pathway of methionine, and that the protein was constitutively produced in the presence of methionine. We have proposed that beta-cystathionase contributes to degenerative changes in bone-forming osteoblast cells by cleaving cystine to thiocysteine, a product which donates sulfur and inactivates essential cellular enzymes. To determine the role of individual amino acids in toxicity and catalysis of cystine, the *B. avium metC* gene was subjected to oligonucleotide-directed, site-specific mutagenesis. The *metC* gene was introduced into pET-11d, resulting in a 30-fold increase in production of betacystathionase in *E. coli* SA2821 as compared to *B. avium* 197. Substitution of alanine for lysine at position 214, the putative co-factor (pyridoxal 5'-phosphate) binding site, resulted in a 95% reduction in beta-cystathionase activity, indicating that lysine residue 214 is involved in catalysis but is not essential for enzyme activity. Replacement of cysteine residues 117 and 309 with alanine resulted in >90% reduction in enzyme activity while substitution of cysteine residues 88 and 279 had a minor effect on enzyme activity. In order to develop plasmid vectors for genetic transfer and mutagenesis, native plasmids were isolated from *fusobacterium* species. It was determined that *F. nucleatum* and *F. russii* contain one plasmid while *F. necrogenes* contains two plasmids. We were unable to isolate plasmids from *F. periodonticum, F. mortiferum, F. ulcerans, F. sulci, F. necroforum, F. varium, F. perfoetans, F. gonidiaformans, F. simiae,* or *F. naviforme*. A 10 kb plasmid from *F. necrogenes* was isolated and characterized by restriction enzyme analysis. This plasmid will serve as the basis for shuttle vector development in future studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00514-04 LME

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Anthrax Toxin - A Model for Bacterial Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Leppla, Stephen H.	Research Chemist	LME, NIDR
Klimpel, Kurt R.	Staff Fellow	LME, NIDR
Arora, Naveen	Visiting Associate	LME, NIDR
Gordon, Valery	Staff Fellow	LME, NIDR
Uchida, Ikuo	Visiting Fellow	LME, NIDR
Stepanov, Alexey S.	Visiting Fellow	LME, NIDR
Haley, Sheila	Microbiologist	LME, NIDR

COOPERATING UNITS *(if any)*

Vollum Institute, Oregon Health Sciences University (G.Thomas)
Department of Microbiology, University of Massachusetts (C.B. Thorne)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.07	3.67	1.40

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project characterizes the structure and function of bacterial protein toxins to determine how toxins contribute to bacterial pathogenesis. Sensitive eukaryotic cells are studied to identify subcellular systems with which the toxins interact. (A) In order to intoxicate cells the anthrax toxin protective antigen (PA) must be activated by cleavage after the amino acid sequence RKKR. Mutant animal cell lines were derived that are deficient in proteases that normally activate PA. Several of these mutant cell lines appear to lack the protease furin, which is known to recognize sequences having R at the -1 and -4 positions, as in the sequence RAAR. Thus, the mutant cells are resistant to a PA variant having the cleavage sequence RAAR and to Pseudomonas exotoxin, which requires proteolytic activation at the sequence RQPR. Other mutant cell lines were obtained that are resistant to PA variants having the sequence SSRR. Evidence suggests these cell lines lack a protease that recognizes the RR sequence. (D) Improved methods were developed for production and purification of fusion proteins containing amino acids 1-254 of the anthrax toxin LF protein and cytotoxic catalytic domains from other toxins. These proteins are efficiently translocated by the anthrax toxin PA protein to the cytosol of animal cells where they cause cell death. The fusion proteins are being developed as one component of therapeutic, cell-type specific cytotoxic agents. (C) Strong evidence was obtained that the anthrax toxin LF protein is a metalloprotease. A sequence HEXXH, shared by all metalloproteases, was found in the LF sequence. Site-directed mutagenesis to change either of the His residues to Ala completely destroyed toxicity. Furthermore, a number of protease and peptidase inhibitors protected cells against the toxin. The results have wider interest because tetanus toxin and the botulinum neurotoxins were recently shown to be metalloproteases. (D) A Bacillus anthracis gene was identified that is needed to stimulate anthrax toxin synthesis. Sequencing showed the gene encodes a protein of 476 amino acids. No similar proteins were found in existing databases so this gene may be representative of a new class of regulator.

Principal Investigator

Fields, Raymond	Chemist	LME, NIDR
Keith, Jerry M.	Chief, LME	LME, NIDR

Cooperating Units

Laboratory of Cellular and Molecular Biology, NCI, NIH (S. Aaronson)
Laboratory of Molecular Biology, NCI, NIH (D.J. Fitzgerald)
Department of Microbiology and Molecular Genetics, Harvard Medical School (R.J. Collier)
Laboratory of X-ray Crystallography, Dana-Farber Cancer Institutue (R.C. Liddington)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00518-04 LME

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Detoxified Pertussis Toxin for Acellular Whooping Cough Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Keith, Jerry M.	Chief, Laboratory of Microbial Ecology	LME, NIDR
Nicholls, Peter	Visiting Associate	LME, NIDR
Merkel, Todd	Staff Fellow	LME, NIDR
Fields, Raymond	Chemist	LME, NIDR

COOPERATING UNITS *(if any)*

NIH, Tokyo Japan (H. Sato); Washington University, St. Louis, MO (R. Curtiss III); University of Missouri, Columbia, MO (C. Parker)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20822

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	2.00	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Whooping cough is caused by an infection of the respiratory tract with *Bordetella pertussis* bacteria. This disease is effectively controlled by the current vaccine which consists of killed whole *B. pertussis* cells. Though efficacious, the present vaccine produces unacceptable side effects. The major protective antigen in whooping cough vaccines is pertussis toxin. Clinical trials of acellular pertussis products strongly indicate that pertussis toxin will be a necessary and perhaps sufficient component of any new vaccine. Chemically "inactivated" pertussis toxin vaccines have been produced with reduced side effects and reasonable efficacy, however, residual activity may exist. Using site-specific DNA mutagenesis, we modified an *E. coli* subclone of the pertussis toxin S1 subunit and then used these constructs to replace the chromosomal copy of the toxin gene in *B. pertussis* strain 3779. The resulting new strain produces a fully genetically detoxified form of pertussis toxin which is strongly immunoprotective and can be used as a vaccine antigen without chemical inactivation. Molecular studies are currently underway in our laboratory to develop high yield *B. pertussis* strains to enhance expression of pertussis toxin for use in acellular and conjugate vaccine manuacture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00537-03 LME

PERIOD COVERED

Protein Virulence Factors of Bacterial Periodontal Pathogens

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

October 1, 1992 to September 30, 1993

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Stephen Leppla	Research Chemist	LME, NIDR
Kurt Klimpel	Staff Fellow	LME, NIDR
Naveen Arora	Visiting Associate	LME, NIDR
Ikuo Uchida	Visiting Fellow	LME, NIDR
Valery Gordon	Staff Fellow	LME, NIDR
Sheila Haley	Microbiologist	LME, NIDR
Jerry M. Keith	Chief, Lab. of Microbial Ecology	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.55	0.45	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to identify new virulence factors of oral bacteria. This search concentrates on proteins having catalytic activities harmful to host cells and tissues. These proteins will be classified as protein toxins if they have the ability to damage cell membranes or to penetrate to the cytosol and disrupt metabolic processes. In the current year, most of the activity of this project evolved into more detailed examinations of individual toxins, with some of the results being reported in other project descriptions. Effort under this project provides support for molecular biology procedures and the logistical and technical support for the detailed studies of individual toxins, while continuing to develop systems capable of detecting and analyzing new virulence factors.

A fusion protein system derived from components of anthrax toxin was used to internalize the osteotoxin derived from *Bordetella avium*, beta-cystathionase, into cultured cells. Fusions with the anthrax toxin retained enzymatic activity and were toxic to cells. These fusion proteins can be used to determine if the osteotoxin is more potent on the plasma membrane or in the cytosol.

Mouse macrophage cell line systems were developed for study of phagocytosis of toxin-producing bacteria. Methods for measuring bacterial uptake, growth, and for killing of the macrophages were examined. This system will be used to search for other virulence factors involved in bacterial invasion and persistance in tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00564-02 LME

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics and Toxic Mechanism of Leukotoxins of Pathogenic Oral Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Carolyn L. Bouma	Staff Fellow	NIDR, LME
Elisabeth Holmes	Biological Aide	NIDR, LME

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	0.9	0.30

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pathogenic oral bacteria are often associated with the progression of oral diseases, such as periodontitis. The Gram-negative bacterium *Actinobacillus actinomycetemcomitans* (*Aa*)colonizes periodontal sites and produces a leukotoxin that is likely to play an important role in periodontitis. Understanding the role of the *Aa* leukotoxin and other virulence factors in oral disease requires knowledge of the conditions under which such factors are produced, and how they act.

Because of its probable role in periodontal disease, we have initiated studies of the genetics and mechanism of cytotoxicity of the *Aa* leukotoxin, LktA. *LktCA* has been isolated from *Aa* ATCC 29524 by PCR amplification, and we have developed fluorescent and isotopic assays for cytotoxicity. We have shown that cytoplasmic extracts of recombinant *E. coli* carrying *Aa lktCA* contain a protein that is cytotoxic to a human pre-monocyte cell line. The protein has been partially purified by S-sepharose chromatography, and is stabilized in the presence of denaturing agents (4M urea, 1% CHAPS). We are using recombinant DNA techniques to generate a histidine-labeled protein, which will be purified by Ni-NTA chromotography and used to prepare an anti-LKtA antibody. The sites of transcription initiation for *Aa lktCA* have been identified. We have identified Ca^{2+} as an extracellular factor that modulates activity of the *lkt* promoter in *Aa* strain JP2. We propose to use Northern hybridizations to investigate other environmental factors that might affect LktA synthesis (temperature, CO_2, and others).

We plan to use DNA hybridization as a tool to identify other oral bacteria that might produce leukotoxins. Genomic DNA from normal oral flora as well as organisms frequently isolated from diseased individuals will be included in this survey.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00557-02 LME

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Specific Interactions of Bacteria with Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Sandberg, Ann L.	Chief, Microbial Receptors & Pathogenesis Sec.	LME, NIDR
Sutphin, Michelle	Biologist	LME, NIDR
Lee, Si Young	Visiting Fellow	LME, NIDR
Ruhl, Stefan	Visiting Associate	LME, NIDR
Cisar, John O.	Research Microbiologist	LME, NIDR
Takahashi, Yukihiro	Visiting Fellow	LME, NIDR
Bryant, Joe	Chief, ACU	LME, NIDR

COOPERATING UNITS *(if any)*

Dr. Mike Eckhaus, VRP, NCRR, NIH; Jennie Owens, VRP, NCRR, NIH

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbial Receptors and Pathogenesis Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.33	3.33	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Gal/GalNAc reactive lectin of *A. naeslundii* WVU45 and the sialic acid reactive lectin of *S. gordonii* DLI recognized the same glycoprotein receptor on polymorphonuclear leukocytes (PMNs). Although the streptococcal lectin bound to the native sialylated receptor, binding of the actinomyces lectin required prior exposure of the appropriate saccharides by sialidase, an enzyme produced by these bacteria. Expression of this receptor was influenced by cell differentiation as indicated by enhanced binding of either bacteria during DMSO-induced differentiation of HL60 cells toward PMNs. This receptor was also subject to differentiation-dependent processing. The apparent molecular weight of the receptor on undifferentiated cells was 150 kD but shifted to that found on circulating PMNs (130 kD) following exposure of the HL60 cells to DMSO. Recognition of this PMN receptor by either of these bacterial lectins probably contributes to inflammation since both stimulated the production of superoxide anions as well as the release of PMN granule contents. Both bacteria were subsequently phagocytosed by the PMNs and the actinomyces, but not the streptococci, were killed. Six additional strains of *S. gordonii* were also phagocytosed by PMNs and, like DL1, two strains remained viable. Two strains were slightly susceptible to lectin-mediated destruction by PMNs but the remaining two strains were completely resistant. In a rat model of endocarditis the four strains that were resistant or only slightly susceptible to destruction by PMNs induced severe infection of previously traumatized aortic valves whereas the two strains that were destroyed by PMNs produced only mild infection. The production of endocarditis by these six strains of streptococci failed to correlate with aggregation of platelets, attachment to fibrin-platelet matrices or binding to fibronectin, laminin or fibrinogen. A major determinant of virulence for the production of endocarditis by this group of streptococci, therefore, appears to be resistance to lectin-mediated killing by PMNs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00571-01 LME

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Regulation of Genetic Competence in Oral Streptococci

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lunsford, R. Dwayne	Staff Fellow	LME, NIDR
	London, Jack P.	Research Microbiologist	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this new project is to study genetic competence (competent for genetic transformation) induction in *Streptococcus gordonii* as a model system for global genetic regulation in the oral strptococci. Standard molecular genetic techniques are being utilized to isolate genes specifically induced at competence and to characterize the gene products responsible for DNA uptake and processing. This activity will provide a set of developmentally induced loci with which to begin a systematic investigation of specialized gene expression in this genera.

A second major aim of the project is to determine what role genetic transformation may play in the horizontal transfer of genetic information within the streptococcal compartment of the oral microbiota. These studies will determine whether competence has any relevance to antigenic variation and the overall genetic fitness for oral colonization by this group of organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00601-01 LME

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

HIV-targeted cytotoxic proteins derived from anthrax toxin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Leppla, Stephen H.	Research Chemist	LME, NIDR
Klimpel, Kurt	Staff Fellow	LME, NIDR
Gu, Mi-Li	IRTA Fellow	LME, NIDR
Teixeira, Avelino V.	Visiting Fellow	LME, NIDR
Nicholls, Peter J.	Visiting Associate	LME, NIDR
Arora, Naveen	Visiting Associate	LME, NIDR
Gordon, Valery M.	Staff Fellow	LME, NIDR

COOPERATING UNITS *(if any)*

J. Rapapport

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.48	2.03	0.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several unique features of anthrax toxin are being exploited to make novel, cell-specific cytotoxins for killing HIV-1 infected cells. The strategies for killing cells utilize highly-toxic fusion proteins in which the amino-terminal portion of anthrax toxin lethal factor (LF) is genetically fused to the ADP-ribosylation domain of *Pseudomonas* exotoxin A (PE). Delivery of these LF-PE fusion proteins to the cytosol of cells requires the prior binding and proteolytic activation of the protective antigen (PA) component of the toxin. Three separate approaches to targeting cells are being used:

1. The site in PA which must be proteolytically cleaved was replaced by consensus sequences recognized by HIV-1 protease, so that PA will be activated only in HIV-1-infected cells. Four of five mutant PA proteins were cleaved by HIV-1 protease. It is anticipated that these PA mutants will sensitize infected cells to exogenously-added LF-PE fusions.
2. The receptor-binding portion of PA is to be replaced by CD4, IL-2, or single-chain antibodies directed to HIV gp120 or gp41, so that the high efficiency anthrax toxin translocation mechanism will internalize the LF-PE fusions into infected lymphocytes. A model system is being used in which a peptide, EQKLISEEDLN, is fused on the carboxyl-terminus of PA, replacing the normal receptor-binding region of PA. A hybridoma cell line having surface-displayed antibodies to this peptide is expected to bind and internalize the PA-peptide fusion.
3. The gene encoding PA will be transfected into cells under control of transcriptional regulators encoded by HIV-1, so that only infected cells will produce PA and become sensitized to exogenous addition of the LF-PE fusions. To test whether PA produced within cells will be able to internalize LF-PE fusions, well-characterized vectors able to express PA are being transfected into appropriate cultured cell lines.

Principal Investigator

Chi, Angela C.	Biologist	LME, NIDR
Keith, Jerry M.	Chief, LME	LME, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00423-08 LOM

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cloning, Expression and Characterization of Human Autoantigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

M.S. Lan	Senior Staff Fellow	LOM, NIDR
M. G. DeSilva	Visiting Associate	LOM, NIDR
J. Lu	Visiting Fellow	LOM, NIDR
Q. Li	Visiting Fellow	LOM, NIDR
G. Donadel	Visiting Associate	LOM, NIDR
F.P. VanderVegt	IRTA Fellow	LOM, NIDR
A.L. Notkins	Medical Director	LOM, NIDR

COOPERATING UNITS (if any)

Navy Medical Oncology Branch, NCI, National Naval Medical Center; BCDP-Dyn Corp.,/Program Resources, Inc.; Frederick Cancer Research and Development Center

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.2	5.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two novel cDNAs, IA-1 and IA-2, isolated from a human insulinoma subtraction library (ISL-153) were subjected to intensive studies. IA-1 gene was isolated and characterized to be an intronless gene which localized at chromosome 20p11.2 by fluorescence in situ hybridization. We have conducted a study of IA-1 gene expression in a panel of 64 endocrine and non-endocrine human lung cancer cell lines and compared it with two other neuroendocrine markers, chromogranin A and L-dopa decarboxylase. The result indicated that IA-1 is a candidate marker of neuroendocrine differentiation of human lung tumors. IA-1 mRNA was detected by Northern blot analysis in 97% (30/31) of small cell lung cancer (SCLC) cell lines. In contrast, IA-1 mRNA was detected in only 13% (4/30) of non-small cell lung cancer (NSCLC) cell lines. In most of the lung cancer cell lines examined, IA-1 showed high concordance with the other neuroendocrine markers, L-dopa decarboxylase and chromogranin A. The second cDNA, IA-2, was characterized to be a new member of receptor-type protein tyrosine phosphatase which expressed in normal enriched islets and brain tissues. We have also cloned and sequenced the mouse counterpart of IA-2 from a normal mouse brain library. Mouse brain IA-2 revealed 85% and 92% homology to the human insulinoma IA-2 molecule in nucleotide sequence and amino acid sequence respectively. Furthermore, both molecules share 99.7% identify in a stretch of 300 amino acids of intracellular PTP domain.

Professional Personnel, continued

D.A. Trado	Secretary	LOM, NIDR
E.Q. Mange	Editorial Assistant	LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00471-06 LOM

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transgenic Mice as Models for the Study of HIV-1 Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

J.B. KoppS	Senior Research Investigator	LOM, NIDR
A.L. Notkins	Medical Director	LOM, NIDR
P.E. Klotman	Medical Officer	LOM, NIDR
P.E. Ray	Clinical Fellow	LOM, NIDR
J. Dumois	Staff Fellow	LOM, NIDR
L.A. Bruggeman	Senior Staff Fellow	LOM, NIDR
R. Franks	Senior Staff Fellow	LOM, NIDR
J. Rappaport	Senior Staff Fellow	LOM, NIDR

COOPERATING UNITS (if any)

LCP, NCI (M. Sporn)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.80	2.90	1.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transgenic mice provide a unique model system to investigate molecular and cellular mechanisms of HIV-1 pathogenesis. Studies in HIV-transgenic mice from several laboratories, including our own, have helped to clarify the role of HIV-1 gene products in inducing disease independent of opportunistic infection. Our laboratory has developed several mice transgenic for HIV-1 genes. Recent studies have extended the characterization of one of these transgenic lines, pNL4-3:d1443, which contains the viral *ltrs*, the *env* gene and the six accessory genes.

Our work with this mouse model over the past year has focused on three disease processes. First, severe post-natal growth retardation and early mortality characterize the homozygous d1443 mouse, which makes this a model for HIV-associated pediatric growth retardation. Homozygous mice have marked hypoglycemia and reduced liver IGF-1 mRNA expression. Second, both the homozygotes and heterozygotes develop progressive renal disease, characterized by glomerulosclerosis and tubulointerstitial disease, which closely resembles HIV-associated nephropathy. This syndrome affects the majority of d1443 mice, and ultimately 40% of transgenic mice die of uremia. Two growth factors, TGF-β and bFGF, are present in increased amounts in the interstitium of transgenic mice. We propose that TGF-β increases accumulation of extracellular matrix protein and that bFGF stimulates cell proliferation. Third, d1443 mice develop focal epidermal papillomas, both spontaneously and with trauma. Using the appearance of UVB-induced papillomas as a test system, we have shown that oral administration of glutathione suppresses papilloma induction by 80%. Thus the d1443 mouse provides a convenient small animal model with which to investigate HIV-1 pathogenesis, as well as to test novel therapeutic approaches.

Professional Personnel, continued

Bryant, J.	Veterinarian	ACU, NIDR
Weeks, B.	NRSA Fellow	LDB, NIDR
Marinos, N.J.	Bio. Lab. Tech.	LOM, NIDR
Wohlenberg, C.	Microbiologist	LOM, NIDR
Trado, D.A.	Secretary	LOM, NIDR
Mange, E.Q.	Editorial Assistant	LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00534-03 LOM

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Immunoglobulin Genes and their Properties

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

S. Cheung	Staff Fellow	LOM, NIDR
S. Takeda	Visiting Associate	LOM, NIDR
N. Dorfman	Expert	LOM, NIDR
W. Kajiyama	Visiting Associate	LOM, NIDR
R. Sadaie	Senior Staff Fellow	LOM, NIDR
A.L. Notkins	Medical Director	LOM, NIDR
D.A. Trado	Secretary	LOM, NIDR
E.Q. Mange	Editorial Assistant	LOM, NIDR

COOPERATING UNITS (if any)

NIDDK (E.A. Padlan); New York University (P. Casali); Thomas Jefferson University (B. Dietzschold)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.75	4.35	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using human hybridoma technology, we have generated eleven new monoclonal antibodies to rabies virus and autoantigens. These antibodies can be classified into either monoreactive high-affinity or polyreactive low-affinity molecules. One of these antibodies has rabies virus neutralizing activity. Because of the instability of human hybridomas, we have rescued and cloned Fab genes from several antibodies and expressed these genes in E. coli. The bacteria-expressed recombinant Fabs retained their binding specificity. Since the immunoglobulin genes are cloned in bacterial vectors, they can be molecularly manipulated in order to study the structure-function relationship of an antibody molecule. In this regard, we have used mAb 57 as a model antibody to investigate the effects of somatic mutations on its biological functions. Our results showed that a single point mutation in the complementarity-determining region (CDR)-1 can lead to a significant loss of binding activity. Several mutant antibodies are being used for further immunological and biochemical studies.

The biological functions as well as the molecular mechanisms by which polyreactive antibodies interact with many dissimilar antigens have not been determined. In this study, our results showed that CDR-3 of polyreactive antibodies is slightly longer. It has more arginine and aromatic amino acid residues than that of the monoreactive antibodies. Based on this knowledge, we are in the progress of using a PCR-based site-specific mutagenesis method to replace these residues with aliphatic and uncharged amino acids in order to determine whether polyreactivity will be retained. To further investigate the role of CDR-3 in polyreactivity, we plan to generate chimeric antibody molecules containing CDR-3 from polyreactive antibodies that has been grafted onto a monoreactive antibody backbone and assay for their binding specificity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00536-03 LOM

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Polyreactive and Monoreactive Autoantibodies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

G. Sigounas	Visiting Associate	LOM, NIDR
G. Chen	Senior Staff Fellow	LOM, NIDR
G. Donadel	Visiting Associate	LOM, NIDR
N. Kolaitis	Guest Researcher	LOM, NIDR
A.L. Notkins	Medical Director	LOM, NIDR
E. Monell-Torrens	Bio. Lab. Tech.	LOM, NIDR
J. Wheeler	Biologist	LOM, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.9	3.5	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Polyreactive antibodies are natural antibodies capable of binding multiple antigens and are commonly found in normal and diseased individuals. To explore mechanisms involved in polyreactivity, and study other biological properties of polyreactive antibodies we performed in vivo and in vitro studies with human monoclonal and polyclonal antibodies. We found that polyreactive antibodies are able to bind multiple antigens simultaneously. The carbohydrate moieties of immunoglobulins are not associated with polyreactivity. The half-life of polyreactive Abs is significantly shorter compared to its monoreactive counterpart and is isotype independent. Using biotinylated antigens as probes to detect antigen-reactive B cells, we found that the cell populations positively selected have a higher percentage of polyreactive antibody-producing B cells than negatively selected populations, and the difference between them is about 10 fold. Thus, antigen binding might be used as a functional marker for isolating polyreactive antibody-producing B cells. Several studies have shown that the majority of polyreactive antibodies are produced by the CD5 antigen-bearing B cells. To understand the relationship between the expression of the CD5 gene and the production of polyreactive antibodies, we cloned, mapped and determined the exon-intron boundaries of the genomic form of the gene. Two targeting vectors have been engineered to mutate the CD5 gene either at the second or second and third exons in ES cells. The totipotency and ability of ES cells to contribute to the development of the germ line of the animal has been tested by making chimera mice.

Professional Personnel, continued

D.A. Trado	Secretary	LOM, NIDR
E.Q. Mange	Editorial Assistant	LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00562-02 LOM

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of HIV and Gene Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

J. Rappaport	Staff Fellow	LOM, NIDR
P.E. Klotman	Special Expert	LDB, NIDR
L.A. Bruggeman	Staff Fellow	LDB, NIDR
R. Franks	Staff Fellow	LOM, NIDR
J. Kopp	Clinical Fellow	LOM, NIDR
J. Bryant	Veterinarian	ACU, NIDR
A.L. Notkins	Medical Director	LOM, NIDR
M. Richardson	Technician	LOM, NIDR

COOPERATING UNITS (if any)

S. Arya, Laboratory of Tumor Cell Biology, NCI; M. Klotman, Laboratory of Tumor Cell Biology, NCI; A. Hampel, University of Northern Illinois; F. Wong-Staal, University of California San Diego; T. Coffman, Duke University, Durham, N.C.

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.72	1.50	2.22

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are 1) to dissect the regulatory mechanisms responsible for HIV-1 replication and based on these findings 2) to develop molecular based strategies for the treatment of AIDS. To accomplish these objectives, we have used various technologies including the development of transgenic models for HIV-1 pathogenesis, development of transgenic mice with potentially useful gene therapeutic constructs, in vitro assays for viral molecular interference, and in vivo methods to explore factors responsible for HIV mRNA processing and transcription. Our studies have identified tissue-specific cellular factors that regulate viral gene expression and the processing of viral mRNA. We have developed transgenic mouse lines that express potentially "protective genes" including an HIV-targeted ribozyme and a gene that expresses molecular decoys for the transactivator of HIV-1 Tat. We have developed methods to explore natural viral interference properties to develop strategies to inhibit HIV-1 replication. Finally, we have utilized our exisiting transgenic models of HIV-associated nephropathy, AIDS related cutaneous proliferative diseases, and growth retardation in young animals to explore the efficacy of systemic antisense oligonucleotide therapy. Ongoing studies are designed to identify tissue-specific "suppressors" and "activators" that may be useful in controlling viral replication, the continued use of transgenic modeling for pathogenesis and therapy, the identification of DNA transporters and the development of novel molecular therapeutic approaches for the treatment of AIDS.

Professional Personnel, continued

M.C. Myers	Technician	LOM, NIDR
C. Wohlenberg	Microbiologist	LOM, NIDR
N.J. Marinos	Bio. Lab. Tech.	LOM, NIDR
S. Adler	Howard Hughes Student	LDB, NIDR
D.A. Trado	Secretary	LOM, NIDR
E.Q. Mange	Editorial Assistant	LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-25 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Design and Computer Interfacing of Neurophysiological Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, Frederick J. Electronic Engineer (Instru) NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These projects involve the design and construction of electronic and electromechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Projects also include the interfacing of these and other instruments to laboratory computers. Electronic circuit design, microcomputers, and assembly language programming may be used in these instruments or interfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00132-19 NA

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacologic Modulation of Neuroendocrine Responses to Stress and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond A.	Unit Chief	NA NIDR
Gordon, Sharon	Postdoctoral Fellow	NA NIDR
Tahara, Martin	Special Volunteer	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

Cannon, Richard	Section Chief	NHLBI
Gilligan, David	Staff Physician	NHLBI

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.9	.8	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are 1) to evaluate the neuroendocrine responses to surgical stress and inflammation, 2) to determine the analgesic and anti-inflammatory effects of prototype and novel drugs which alter either the synthesis or the receptor activation of neuroendocrine mediators, and 3) to evaluate the clinical utility of these novel drugs in controlled clinical trials.

Previous work on the physiologic function of plasma beta-endorphin and regulation of its release has provided evidence of enhanced release by a variety of stressors, including clinical pain following oral surgery. A recent study attempted to extend this line of investigation to evaluate the relationship between endorphin release and chest pain in patients with coronary artery disease. Plasma levels of beta-endorphin were measured at rest, at the peak of treadmill exercise, and following 10 minutes recovery in 46 patients with chest pain and normal coronaries, with coronary artery disease, and in volunteers with normal coronary arteries and no chest pain. A significant increase in beta-endorphin levels were seen over time but not between groups. Greatest levels were seen during recovery but did differ significantly between patients with chest pain and normal coronary arteries, patients with coronary artery disease, or normals. These data are consistent with previous observations that exercise stress is a sufficient stimulus for the release of pituitary beta-endorphin. The lack of differences between the three groups, however, does not provide any support for the hypothesis that a deficient release of endorphin during exercise occurs in patients with chest pain and normal coronary arteries. Rather the data demonstrate that the magnitude of endorphin release is secondary to the duration of the exercise stress, suggesting that endorphin release in these patient groups in response to physical exercise and associated pain is similar to normals.

Increasing evidence suggests that the nociceptive afferent barrage which can occur during a surgical procedure can activate central processes leading to an increased perception of clinical pain long after the nociceptive input is removed. This hypothesis is being evaluated in the oral surgery model by randomly allocating local anesthesia or placebo anesthesia prior to the surgical removal of third molars with general anesthesia. Demonstration of a difference between groups at 24 and 48 hours, long after the local anesthetic has dissipated, will provide evidence to support the hypothesis that nociceptive afferent barrage produces central plasticity leading to increased postoperative pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00133-19 NA

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assessment of Experimental and Clinical Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NA NIDR
Dionne, Raymond	Research Pharmacologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Max, Mitchell	Physican	NA NIDR
Smith, Wendy	Psychologist	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.45	.65	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The interactive computer-based staircase scaling method was used in two experiments, and the continuous track-ball method also was used in two experiments, one delivering 1.4 sec 40^0 thermal stimuli, the other delivering stimuli ranging from 37 to 53^0 C. A study evaluating memory of clinical pain magnitude was completed. Two additional studies using the interactive method and the track-ball assessment of 1.4 sec stimuli were initiated during this period, and studies validating a clinical pain questionnaire, and memory for neuropathic and cancer pain also were initiated.

The first experiment used the interactive method to examine pain sensitivity in patients with cholestasis, a progressive liver disease. The hypothesis that the pruritus associated with this disease is due to increased levels of endogenous opiates was tested by both examining baseline experimental pain responses and assessing the effect of a challenge by a narcotic antagonist. A final analysis of 20 patients showed no evidence for increased levels of endogenous opiates.

The second experiment examined the effects of imipramine and clonidine on the perception of painful thermal stimuli in 60 patients suffering from cardiac pain but without evidence of coronary artery disease. Imipramine reduced the frequency of painful episodes without altering either the painfulness of the episodes or cutaneous pain sensitivity.

The third study used the track ball method to assess the effects of fentanyl or placebo on the magnitude and duration of pain sensation evoked by 3-sec thermal stimuli of varying intensity. Magnitude and duration were both relted to stimulus intensity, but were independent of each other across subjects. In comparison to placebo, fentanyl reduced both the magnitude and durations of thermally-evoked pain sensations.

The fourth study used track-ball assessment of trains of 49 C^0 stimuli to assess the effects of fentanyl on first and second pain sensations. Initial analysis showed that one-half of the subjects can rate both pain components with this method without any instruction about the existence of these two sensations.

The fifth study used an at home pain measuring device and a physical therapy session to evaluate the accuracy and pliability of retrospective reports of chronic pain magnitude.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00286-14 NA

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Therapeutics for Acute Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dionne, Raymond A.	Unit Chief	NA NIDR
Berthold, Charles	Guest Scientist	NA NIDR
Tahara, Martin	Special Volunteer	NA NIDR

COOPERATING UNITS *(if any)*

Rowan, Janet	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.6	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project consists of a series of clinical trials evaluating the clinical efficacy and safety of experimental therapeutic agents for the control of acute pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. The surgical removal of impacted third molars serves as a model of minor surgery with associate pain and perioperative apprehension. All studies are double-blind with randomly allocated, parallel treatment groups and multiple dependent measures of therapeutic efficacy and clinical safety.

A recent study evaluated the analgesic efficacy of the combination of ibuprofen and oxycodone to define an analgesic combination which results in additive analgesia for the management of pain not responsive to the use of a single agent such as ibuprofen and related drugs. The highest dose of oxycodone (10mg) resulted in additive analgesia in comparison to ibuprofen alone (400 mg) or ibuprofen plus lower doses of oxycodone. This dose also produced a high incidence of undesirable effects such as nausea, vomiting, and drowsiness which would limit its use in ambulatory patients. Demonstration of an additive effect for an opioid-non steroidal anti-inflammatory drug combination, however, provides a basis for the management of severe acute pain with an oral drug combination when alternative treatments have proven inadequate.

A parallel study evaluated the efficacy of nonsteroidal anti-inflammatory drugs applied peripherally at the site of injury. A propietary formulation of ketoprofen was administered directly into third molar extraction sites in comparison to a placebo formulation. Administration of a dose which was 20% of the normal systemic dose suppressed administration of the same dose at the extraction site to oral administration of the gel formulation to demonstrate that the analgesic activity is not due to a systemic effect following absorption from the extraction site. Replication of an analgesic effect would support peripheral administration for achieving greater efficacy and a lower incidence of side effects by minimizing systemic exposure.

A previous study demonstrated that oral administration of a benzodiazepine hypnotic agent produces anxiolytic activity in th oral surgery model comparable to parenteral administration of diazepam. A current study is evaluating if sublingual adminstration of triazolam results in greater efficacy or less psychomotor effects than oral administration of the same dose. Preliminary results suggest that sublingual administration produces greater anxiety relief but without any greater psychomotor impairment than oral administration. These two studies hold promise for the clinical use of a single entity, non-parenteral form of sedation with efficacy comparable to 7parenteral administration but with greater safety.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00288-14 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of the Molecular Response to Noxious Stimulation and Nerve Injury

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ruda, Maryann	Chief, Cellular and Molecular Mechanisms Section	NA NIDR
Allen, Barbara V.	Biologist	NA NIDR
Franklin, Emma L.	Biological Lab Tech. (Electron Microscope)	NA NIDR
De Leon, Marino A.	Staff Fellow	NA NIDR
Besse, Dominique	Visiting Scientist	NA NIDR
Ren, Ke	Visiting Associate	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	1.5	.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research project is designed to identify the neuronal response to noxious stimulation of the periphery and nerve injury. Neurons in the dorsal root ganglion and spinal cord represent the first level of processing of neuronal information from the periphery. Using cellular and molecular techniques it is possible to identify important elements in the neuronal networks that subserve the response to nociception, nerve injury and regeneration. Calcium binding proteins (CBP) play an important role in cellular homeostasis and excitotoxicity related to ionic changes that occur in response to stimulation. Several different CBPs are found in the spinal cord. A comparison of their localization was undertaken in an effort to identify those related to nociceptive inputs or nerve injury. In the dorsal horn, calbindin D-28K (CB) is predominant in laminae I while calretinin (CR) is unique to laminae V and VI and calmodulin (CM) is densly distributed in lamina IX. In lamina II, both CB and CR are similarly distributed. CM which has been hypothesized to be important in the nitric oxide cascade, is not significant in the superficial dorsal horn, an observation in contradiction to that predicted by nitric oxide localization. The laminar localization of each CBP suggests that multiple CBPs are involved in the dorsal horn response to noxious stimulation and nerve injury. Nitric oxide (NO) has been proposed as a novel neuronal messenger involved in either cell protection or excitotoxicity. Because of the instability of NO, localization of the synthetic enzyme, nitric oxide synthase (NOS), with either antibodies, NADPH histochemistry or molecular probes has been used to study NO. Previous work in our laboratory has identified an induction of NOS mRNA in DRG after sciatic nerve cut. We further examined this effect in rats neonatally treated with capsaicin (CAP) to destroy a subpopulation of small-sized nociceptive DRG neurons. CAP is thought to act through a Ca^{2+} dependent excitotoxicity. Our studies demonstrated that most NO containing DRG neurons are capsaicin-sensitive and that the presence of NO does not protect the neurons from capsaicin induced excitotoxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00291-14 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analgesic Mechanisms in the Medullary Dorsal Horn of Behaving Monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas, David A.	IRTA Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Kenshalo, Jr., Daniel R.	Research Biologist	NA NIDR
Williams, Gene M.	IRTA Fellow	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	.9	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There exist both noradrenergic and opioid based systems of pain control. We examined the effects of activating these systems by injecting antinociceptive agents into the medullary dorsal horn (MDH) and examining the ability of monkeys to detect small increases in noxious thermal stimuli. The effects of these agents on facial scratching behavior in separate experiments were also examined. In the detection paradigm, the monkeys were required to detect temperature changes of 0.4, 0.6 and 1.0°C (T2) superimposed on a 46.0°C (T1) stimuli. Consistent with previous research, 10 micrograms of the noradrenergic agonist, ST-91, and also 3 micrograms of the opioid agonist, morphine, each increased the time to detection of the heating stimuli. Both morphine and ST-91 also produced small increases in the monkeys time to detection of innocuous cooling stimuli. Neither morphine nor ST-91 interfered with the detection of visual stimuli, indicating the effects of these agents are not the result of changes in motoric, attentional, or motivational aspects of the monkeys behavior. In separate experiments, morphine administered into the MDH, but not ST-91, was found to produce a great amount of facial scratching behavior. In both the detection and the scratching paradigms, the noradrenergic receptor antagonist, idazoxan (1.0 mg/kg: I.M.) attenuated the effects of both ST-91 and morphine. In the detection paradigm, the opioid receptor antagonist, naloxone (1.0 mg/kg: I.M.) was only effective at attenuating the effects of morphine, not ST-91. These findings demonstrate that the opioid and noradrenergic systems involved in both pain and itch interact. The present data has increased our understanding of how pain control systems interact, which may lead to precise co-activation of opioid and noradrenergic pain control systems in clinical settings. In terms of itch control, these finding indicate that noradrenergic antagonists may be effective antipruritic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00329-12 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Discrimination of Thermal Stimuli in the Monkey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kenshalo, Jr., Daniel R.	Research Biologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Thomas, David A.	IRTA Fellow	NA NIDR
Douglass, Diana	Postdoctoral Fellow	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.63	1.43	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the neural mechanisms that subserve the monkey's ability to detect innocuous cutaneous stimuli (air puffs) delivered to the face. The magnitude of sensations produced by small increases in air puff stimuli was studied with use of a reaction time paradigm. The monkey initiated a trial by pressing an illuminated button. Subsequently air puff stimuli (AP1) of identical intensity were delivered to the face at the rate of one per second. After a variable time period between 4 and 10 seconds, an air puff of higher intensity (AP2) was presented. The subject was required to release the button as soon as the larger air puff stimulus was detected. Detection latency was defined as the time interval between the onset of the large air puff and the release of the button. The monkey's detection latencies to stimuli presented on the face were dependent on the intensity of the AP2. The psychophysical functions obtained from the monkey's face were monotonically related to the intensity of AP2. As the intensity of AP2 increased, the monkey's detection latencies shortened. Neuronal recordings were made from the medullary dorsal horn (MDH) as the monkey performed the psychophysical task. Responses produced by air puff stimuli were examined in low threshold mechanosensitive (LTM) and wide-dynamic-range (WDR) neurons. A subpopulation of WDR neurons was found that encoded the intensity of AP2 stimuli. In addition, a significant correlation was found between the peak neuronal discharge and the monkeys' detection speeds to air puff stimuli for a subpopulation of WDR neurons. Similarly, LTM neurons also encoded the intensity of AP2 stimuli and the magnitude of their discharge was correlated with the monkeys' detection speed. We concluded that the discharge of a subpopulation of WDR and LTM neurons can account for the monkeys' ability to detect innocuous air puff stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00366-11 NA

PERIOD COVERED

October 1, 19923 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analgesic Mechanisms in Patients with Chronic Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Max, Mitchell B.	Physician	NA NIDR
Bayas-Smith, Michael	Clinical Associate	NA NIDR
Park, Karen	NRSA Fellow	NA NIDR
Sethna, Navil	Clinical Associate	NA NIDR
Iadarola, Michael	Senior Staff Fellow	NA NIDR
Gracely, Richard H.	Research Psychologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Bennett, Gary J.	Chief, NPPM Section	NA NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	3.1	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to elucidate the neural mechanisms and principles of treatment of chronic pain syndromes, with particular attention to the drug treatment of pain caused by nerve injury.

Forty-one patients with painful diabetic neuropathy completed a double-blind comparison of transdermal clonidine to placebo. Because previous work had suggested that only about one-quarter of these patients respond to clonidine, we used a novel two-stage study design in which responders in the initial clinical trial were entered into a second trial, aimed at confirming that they were consistent responders. In the overall group of 41 patients, the treatment difference between clonidine and placebo was not significant, but the 12 apparent responders from the first trial showed a 20% reduction in pain with clonidine, relative to placebo, during the second clinical trial. Brief sharp and shooting pains were more likely to respond to clonidine. The results support the hypothesis that there is a subset of patients with painful diabetic neuropathy who benefit from systemic clonidine and illustrate the value of an "enriched enrollment" clinical trial design in studying pain syndromes which may have diverse underlying mechanisms.

Because of growing evidence from animal studies that neuropathic pain may be mediatd by spinal cord neurons activated via NMDA channels, the NMDA antagonist ketamine is being studied in two conditions: chronic pain in patients with nerve damage or reflex sympathetic dystrophy, and experimental pain in normal volunteers, consisting of hyperalgiesia of the skin briefly induced by intradermal injection of capsaicin. Ketamine reduced capsaicin-evoked pain and hyperalgesia in volunteers, and relieved pain and hyperalgesia in 6/8 patients with chronic neuropathic pain. Therapeutic effects only occurred at doses that also caused cognitive impairment, however.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00413-08 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Experimental Neuropathy of Peripheral Nerve in Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bennett, Gary J.	Chief, NP&PM Section	NA NIDR
Tal, Michael	Visiting Associate	NA NIDR
Nachemson, Ann	Special Volunteer	NA NIDR
Johansson, Arne	Special Volunteer	NA NIDR
Xiao, Wenhau	Visiting Fellow	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain and Pain Measurement Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.35	1.15	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An animal model of painful peripheral neuropathy is produced in the rat by a chronic constriction injury to the sciatic nerve. Animals with this nerve injury have behavioral symptoms that indicate disordered pain sensations like those seen in human syndromes. In particular, the rats have hyperalgesia to thermal and mechanical stimuli, allodynia (pain from normally innocuous stimuli) to touch, and spontaneous pain (or dysesthesias). The nerve injury is known to cause transsynaptic degeneration in small, presumably inhibitory, interneurons in laminae I-III, and this is believed to be due to a NMDA receptor-mediated excitotoxic effect of spontaneous discharge from the damaged primary afferent neurons. Recent work shows that an ordinary surgical incision (without intentional nerve damage) has a similar effect. In this case, the functional impairment of the damaged cells may contribute to postoperative pain and tenderness. Dextrorphan, an NMDA blocker, has been shown to block the rats' heat-hyperalgesia, while having little or no effect on mechano-hyperalgesia and -allodynia. Morphine has a different effect: reduction of mechano-hyperalgesia and -allodynia with little or no effect on heat-hyperalgesia. Perineural corticosteriod treatment produces a marked deficit in heat-hyperalgesia, but with little effect on mechano-hyperalgesia and -allodynia. These data indicate the potential usefulness of morphine, perineural corticosteriods, and NMDA blockers in the treatment of neuropathic pain and support our hypothesis that the different kinds of abnormal pain symptoms seen in the clinic may be caused by different pathophysiologic mechanisms; thus indicating the potential need for combination drug treatments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00414-08 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CNS Neurotransmitter Regulation During Peripheral Inflammatory States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Iadarola, Michael J.	Research Pharmacologist	NA NIDR
Gu, Jun	Visiting Fellow	NA NIDR
Messersmith, Donna M.	IRTA Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Stephenson, Christopher G.	Biologist	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.95	2.8	1.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on the transcriptional control of the dynorphin gene, which codes for the dynorphin family of opioid peptides. We have shown that, during peripheral inflammation, dynorphin gene expression is greatly increased in spinal cord neurons where it plays a role in modulating chronic pain. Transient transfection data using in vitro cell lines indicates that the dynorphin gene is activated via the cAMP second messenger system, less so by nerve growth factor, and minimally by the phosphatidyl inositol pathway. These results imply that a transmitter in primary afferent neurons stimulates adenylate cyclase in second order neurons. Thus, some of the neuronal hyperexcitability changes that accompany chronic pain may be maintained by cAMP-dependent events and may be counteracted by increased release of dynorphin peptides. We have functionally characterized the region surrounding the dynorphin AP-1-like (DAP) site with transient transfection assays. This site was previously characterized biochemically as a site that binds several transcription factor protein complexes, one of which is the Fos-containing AP-1 complex. A 41 bp synthetic oligonucleotide centered on the DAP element and one containing a 2 bp mutation, were placed directly upstream of the dynorphin minimal promoter. Cells stimulated with forskolin, produced a dose-related increased (up to 150-fold) in chloramphenacol acetyl transferase gene expression which was nearly eliminated by the mutation. The element is located at -1546 from the transcription start site. With longer constructs only those that contain the DAP element display forskolin induction. Through examination of additional control elements we cloned a protein (UreB1) which specifically binds at bases -208 to -216. Phosphorylation of UreB1 at a tyrosine kinase consensus enhances binding and in vitro transcription. UreB1 is one of the few factors identified that utilize tyrosine phosphorylation to control activity. These studies elucidate the pivotal role of the spinal dynorphin system in pain mechanisms and may provide new avenues for the pharmacotherapy of pain and insights into chronic opioid use and tolerance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00440-07 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology of Experimental Hyperalgesia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ren, Ke	Visiting Associate	NA NIDR
Williams, Gene M.	IRTA Fellow	NA NIDR
Thomas, David A.	IRTA Fellow	NA NIDR
Ruda, M.A.	Chief, CMM Section	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.8	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To search for new and improved therapies for pain, a series of experiments was undertaken to examine the pharmacological mechanisms underlying the hyperalgesia following peripheral nerve or tissue injury. We tested the possibility that nerve growth factor (NGF) may block behavioral signs of hyperalgesia and allodynia in rats receiving chronic constriction injury (CCI) of the sciatic nerve. From post-operative day (POD) 1-3, thermal and mechanical hyperalgesias on the ipsilateral hindpaw developed. When NGF infusion via an osmotic pump was started immediately after the CCI, thermal hyperalgesia was significantly reduced or abolished from POD 5 up to the end of the test period (POD 42), when compared with rats receiving vehicle or no infusion. CCI-induced mechanical hyperalgesia was also abolished by NGF infusion. Delayed infusion of NGF (POD 4) failed to block thermal hyperalgesia. Infusion of NGF had no significant effect on paw withdrawal latency of the rats that had no CCI. Our preliminary results showed that, chronic infusion of a nitric oxide synthase antagonist also significantly attenuated thermal hyperalgesia in CCI rats. We also studied the involvement of N-methyl-D-aspartate (NMDA) receptors in the mechanical hyperalgesia that follows tissue inflammation. Following inflammation of the rat hindpaw, a state of mechanical hyperalgesia was induced. The intrathecal (i.t.) administration of the NMDA receptor antagonists significantly increased mechanical threshold and reduced response duration in the inflamed hindpaw, but had no effect on the non-inflamed paw. Substance P has been proposed as an important mediator for nociception at the first sensory synapse. Selective non-peptide substance P receptor antagonists WIN 51708 and CP 96,345, when injected i.t., produced a maximum of 80% and 84% reduction of behavioral hyperalgesia in inflamed rats, respectively. CP 96,345 was found 10 times less potent than WIN 51708. By using drugs in combination, it may be possible to affect a more clinically effective analgesia than by using single agents alone. Our results suggest that, in inflammation-induced hyperalgesia, the combination of an opioid agonist and an NMDA receptor antagonist produces a supra-additive antihyperalgesic effect; a combination of NMDA and substance P receptor antagonists produces an additive effect. These results suggest that neurochemical alterations contribute to the development of behavioral hyperalgesia in animal models of peripheral nerve or tissue injury. A rational employment of different agents targeting at different receptor systems may have therapeutic value in the treatment of chronic pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00509-04 NA

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of neuropathic pain in human

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gracely, Richard H.	Research Psychologist	NA NIDR
Bennett, Gary	Section Chief	NA NIDR
Byas-Smith, Michael	Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Max, Mitchell	Physican	NA NIDR
Smith, Wendy	Psychologist	NA NIDR

COOPERATING UNITS *(if any)*

Rabinovitz, Elaine	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.55	1.15	.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New findings confirm that many features of neuropathic pain syndromes, including spontaneous pain, mechanical allodynia (pain evoked from stimulation of Aβ low threshold mechanoreceptor (LTM) afferents), and cold hyperalgesia, are found also after experimental intradermal injections of capsaicin, an active ingredient in chili pepper. AβLTM-mediated allodynia was supported by differential tourniquet cuff blocks in 4 patients; allodynia and touch sensations were abolished concurrently. In 2 patients we observed spread of spontaneous pain and mechano-allodynia from the site of injury at a lower extremity to include both the contralateral extremity and upper extremities. These symptoms were attenuated or abolished by elevation of the affected extremity for a period of 30-40 minutes, suggesting a central mechanism maintained by a peripheral generator in the affected extremity that is sensitive to changes in local perfusion. Large doses, up to 1000 g, resulted in a long period of 30 min to several hours in which mechano-allodynia was present in the absence of spontaneous pain, suggesting that in the initiation phase the altered central processing responsible for mechano-allodynia can exist autonomously, i.e. in the absence of ongoing peripheral input. This autonomous processing has not been observed in patients. During this autonomous period, interventions such as partial or complete occlusion of circulation produced an intense, widespread spontaneous pain which was not increased by stimulation of sympathetic activity. This pain also was found if the limb was exsanguinated before the cuff block, and it was attenuated if the nociceptive drive was blocked by infiltration of local anesthetic at the injection site, or after a regional block of the arm at the elbow. These results support the hypothesis that a peripheral injury initiates central processes resulting in spontaneous pain and evoked abnormalities such as mechano-allodynia and cold hyperalgesia. At the time of injury, these proceses may exist independently of peripheral input, although putative sources of further input (e.g. tourniquet cuff) can exacerbate symptoms, such as rekindling spontaneous pain. In the maintenance phase after injury (as observed in patients), the central process may expand to include body sites distant from the injury, however this expanded altered processing appears to be maintained dynamically by input from the injury focus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00526-03 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of genes regulated during neuronal injury and nerve regeneration

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

DeLeon, Marino	Staff Fellow	NA NIDR
Nahin, Richard	Staff Fellow	NA NIDR
Ruda, Maryann	Chief, CMM Section	NA NIDR
Allen, Barbara	Biologist	NA NIDR
Molina, Carlos	NRSA Fellow	NA NIDR
Franklin, Emma	Biologist (Lab Tech)	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	1.85	1.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We studied the regulation of the transcription factors c-*jun*, *junB* and *junD* after sciatic nerve transection. RNA blot analysis identified that c-*jun* mRNA was dramatically induced after sciatic nerve transection. Immunobloting experiments showed that protein extract from dorsal root ganglia ipsilateral to nerve cut contained higher levels of Jun protein than the contralateral control DRG. The increase in protein and c-*jun* mRNA was not restricted to an exclusive-sized population of neurons but rather both *in situ* hybridization studies and immunocytochemical analysis identified neurons of different sizes which showed the increase. The *JunB* mRNA and protein were also induced after transection (two-three fold by day 1). *JunD* mRNA and protein exhibited a high constitutive level of expression in control DRG which persisted after sciatic nerve transection. DNA mobility shift experiments demonstrated that Jun transcription factors bind to both the AP-1 and the CRE sites. The experiments identified a DNA-protein complex specific to the AP-1 binding site. This complex was increased in the ipsilateral as compared to contralateral DRG extracts. The amount of DNA-protein complex was reduced by antisera directed against the protein products of c-*jun*, *junB* and *junD*, but was not altered when treated with a Fos antibody. These data suggest that the Jun family of transcription factors may play an important role in neuronal injury and nerve regeneration. A second area of research characterized a novel cDNA, DA 11, whose expression is regulated after sciatic nerve transection. The cDNA DA 11 was discovered during the screening of a cDNA library made from RNA extracted from DRG three days after sciatic nerve crush. The DA 11 cDNA was sequenced and our analysis showed that: (a) DA 11 encodes a lipid binding protein; (b) DA 11 mRNA is induced in the dorsal root ganglia after sciatic nerve cut or crush; (c) The higher levels of DA 11 mRNA in the DRG as compared with other tissues suggest that the protein product may play an important role in DRG neurons; (d) the up-regulation of DA 11 mRNA in the DRG after sciatic nerve transection suggests that it may play an important role during neuronal injury and/or nerve regeneration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES – PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00532-03 NA

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathophysiology of Chronic Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

Dionne, Raymond	Research Pharmacologist	NA NIDR
Reid, Kevin	Staff Fellow	NA NIDR
DeNucci, Donald	Visiting Scientist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS (if any)

Frank, Joseph	Director, NMR Center	DRD CC
Rosenbaum, Lola	Physical Therapist	DRM CC
Lord, Dorothy	Physical Therapist	DRM CC

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is attempting to better characterize the pathophysiology of chronic facial pain through a series of clinical investigations. A recently completed study evaluated the use of iontophoretic drug administration as a method for achieving high therapeutic levels of drugs in the temporomandibular joint (TMJ) without systemic administration. A standard drug and dose normally administered by iontophoresis, 0.4% dexamethasone in a 4% lidocaine vehicle, was compared to saline placebo for pain referrable to the TMJ. Subjects completed a battery of analgesic questionnaires and measures of mandibular range of motion (vertical, lateral, and protrusive movements) prior to three applications by iontophoresis of either drug of placebo separated by 48 hours. Both dexamethasone and placebo produced a significant reduction in pain scores from baseline following the first two treatments. No difference, however, was seen between groups for pain report or mandibular range of motion. These data indicate that iontophoretically applied dexamethasone may be no more effective than saline placebo in providing pain relief for patients with temporomandibular joint pain. This observation suggests that iontophoretic drug administration to the TMJ may not be an effective method for administering investigational agents or prototypic drugs to investigate the pathophysiology of chronic facial pain originating from the TMJ and associated structures.

A second study is evaluating the use of pain pressure thresholds (PPT) measured by a pressure algometer to quantify pain in the masseter and temporalis muscles in patients with chronic myogenic facial pain. Pain pressure threshold in the masseter and anterior temporalis muscles in patients with well defined myogenous orofacial pain were compared to asymptomatic controls. No significant PPT differences were found between the side indicated by the patient as most painful and the less painful side, supporting theories of centrally-mediated pain. Mean PPTs in patients differed over the four sessions which is consistent with reports of fluctuating levels of pain in patients with TMD. Within and between session reliability were high for patients and controls. These data provide evidence for the utility of pressure algometry as a tool for quantifying subjective pain symptoms of TMD patients in future studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00556-02 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuropeptide Interactions with Excitatory Synapses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Caudle, Robert M.	Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this study is the pharmacology and physiology of neuropeptides in the central nervous system. Experiments were carried out to A) characterize the effects of the endogenous opioid peptide dynorphin on hippocampal pyramidal cells and spinal neurons and B) characterize the effects of mu opioids on a newly discovered p300 like potential in the rat hippocampal slice preparation. In the CA3 region of the guinea pig hippo-campus we discovered that dynorphin has dual effects on NMDA synaptic currents. At low concentrations the NMDA current is increased and at high concentrations the current is decreased. The inhibitory effect is mediated by the kappa2 subtype of opioid receptor. This is the first demonstration of a physiological function for this receptor. The significance of this discovery is that NMDA receptors are known to mediate chronic pain in the spinal cord and that kappa2 receptors and spinal NMDA receptors are found in the superficial spinal dorsal horn. Thus, the kappa2 receptors represent a novel target for the inhibition of NMDA receptors in the treatment of pain. The receptor mediating the excitatory effect of dynorphin has not been determined yet. Future study of the excitatory action should lead to another novel target for pain management. An in vitro rat spinal cord slice preparation was developed. We have found that low concentrations of dynorphin increase the NMDA current in this preparation. High concentrations have not yet been tested. Future work in the spinal cord preparation will focus on the kappa2/NMDA interaction. We have discovered an extracellular potential in the CA1 region of the rat hippocampal slice that resembles the p300 in human EEG studies. Addition of mu selective agonists reduces the amplitude of this potential. The amplitude of the p300 is highly correlated with the ability of a subject to perceive information and pain management often exploits drugs that alter perception. Thus, this p300 like potential offers an in vitro method to study drugs that may alter perception of painful stimuli. Future work will focus on pharmacological characterization of this potential.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00599-01 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Somatosensory studies of pain and pain control measured with PET and fMRI

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Iadarola, Michael J.	Research Pharmacologist	NA	NIDR
Max, Mitchell	Chief, Clinical Trials Unit	NA	NIDR
Kenshalo, Jr., Daniel R.	Research Biologist	NA	NIDR
Gracely, Richard	Research Psychologist	NA	NIDR
Bennett, Gary J.	Chief, NPPM Section	NA	NIDR

COOPERATING UNITS *(if any)*

Berman, Karen F.	Chief, PET Imaging Unit	CBDB	NIMH
Balaban, Robert	Chief, Laboratory Cardiac Energetics	LCE	NHLBI
Wen, Han	Guest Researcher	LCE	NHLBI

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this clinical research effort is to understand the processing of pain in normals and in patients with neuropathic and/or chronic pain conditions. High resolution positron emission tomography and radiotracer techniques were used to assess regional brain activity via blood flow changes throughout the entire brain of normal humans and neuropathic pain patients. This allows us to determine if abnormalities exist in the neuropathy patients and compare these observations to experimental pain in normals. Over the past 8 months we have completed 19 Oxygen-15 water blood flow PET studies on 13 normal subjects, 4 patients with unilateral chronic neuropathic pain, 1 with bilateral neuropathy and 1 with unilateral post-herpetic neuralgia (PHN) in the trigeminal distribution. In the thirteen normals we determined the pattern of regional activation due to acute pain induced by subcutaneous injection of capsaicin and pain due to allodynia that arises subsequent to capsaicin injection. A wide variety of limbic and somatosensory regions both cortical and subcortical were activated in the two conditions compared to either resting state or normal light brushing. These data indicate that specific additional circuits are recruited in the allodynia condition compared to acute pain. Preliminary analysis of the scans of the patients indicates a consistent alteration in thalamic activity. We observe, in 4 out of 4 patients with unilateral neuropathy, a decrease in thalamic activity on the side of the brain the receives input from the neuropathic limb (3 left lower limb patients and 1 right lower limb patient). The observation of a decrease suggests that the thalamus is an important site for neural modulation of pain in chronic conditions. Functional magnetic resonance imaging (fMRI) is a new technique for studying regional brain activity without radioactive tracers. In collaboration with the Laboratory of Cardiac Energetics, NHLBI, we have begun to apply this method to studying pain processes in cerebral cortex of normals. A spoiled gradient recalled echo flash sequence and a quadature volume coil have been developed to obtain serial functional images of transaxial brain slices during noxious thermal stimulation at high anatomical resolution. The fMRI sequence is supplemented by two anatomical images that provide (a) high resolution grey matter /white matter/CSF anatomy and (b) an angiogram for blood vessel localization. Blood flow changes related to pain have been reproducibly imaged in primary somatosensory cortex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00600-01 NA

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular analysis of the neuronal response to pharmacological treatments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Besse, Dominique	Visiting Fellow	NA NIDR
Ruda, Maryann	Chief, CMMS	NA NIDR
Ren, Ke	Visiting Associate	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.45	1.0	.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies are designed to investigate the regulation of gene expression following pharmacological treatments that alter the response to noxious stimulation of the periphery. We are particularly interested in gene regulation occuring in the dorsal root ganglia (DRG) which contain the cell bodies of primary afferent neurons, and in the spinal cord, where the first inhibitory modulation of nociception occurs. Two series of studies have been initiated. In the first series of experiments, using RNA blot analysis of the spinal cord, morphine treatment was found to inhibit the inflammation-induced increase in c-*fos* mRNA, prodynorphin and, to a lesser extent, proenkephalin mRNAs. These preliminary studies provide evidence that this methodology can be used to examine pharmacological effects on gene expression. The morphine-induced reduction of opioid mRNA expression after inflammation-induced hyperalgesia further support an action of morphine at spinal levels. The parallel decrease in c-*fos* mRNA provides additional support for a relationship between Fos transcription factor and opioid mRNA regulation. The second series of experiments addressed chronic morphine treatment and the induction of tolerance. Morphine often remains the only drug able to relieve persistent pain although the risk of tolerance is a major clinical concern. Thus, it is important to understand the mechanism of such phenomena. Our initial experiments looked at the effects of morphine and the morphine antagonist naltrexone on transcription factor mRNA regulation in the DRG. In rats treated intrathecally with morphine (30 μg/kg/hr) for 4 days, RNA blot analysis identified an initial up-regulation in the expression of c-*jun* mRNA compared to saline-treated rats. Behaviorally, the animals were hypoalgesic to a radiant heat stimulus. In morphine tolerant rats, naltrexone administration produced withdrawal and led to an increase in c-*jun* mRNA expression in the DRG. These data, suggest that some genes responding to Jun transcription factor may have their expression regulated in response to long term morphine administration and withdrawal. Such changes could be part of the mechanism or the consequence of tolerance to morphine.

Research Projects

Office of the Director

Epidemiology & Oral Disease Prevention Program

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00567-02 EPI

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oral Physiology of Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Streckfus, Charles F.	Senior Staff Fellow	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Brunelle, Janet A.	Statistician (Health)	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Albertini, Tullio	Spec. Asst. Prog. Management & Prof. Affairs	EODPP, NIDR
Lipton, James A.	Spec. Asst. Scientific Development	EODPP, NIDR
Oldakowski, Richard A.	Chief, SPU, ASDSB	EODPP, NIDR
Walczak, Cynthia A.	Computer Scientist	EODPP, NIDR

COOPERATING UNITS *(if any)*

NIA, FSK/JHU

LAB/BRANCH

Office of the Director

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.21	0.94	0.27

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The oral research component of the Baltimore Longitudinal Study of Aging (BLSA), since its inception in 1978, has been designed to evaluate the physiological and pathological factors that influence the oral health and function of individuals of different ages. Currently, the EODPP, is developing plans to broaden the scope of research to include studies of alveolar bone loss in the oral cavity, the detection and application of oral molecular biological markers for systemic disease, and an expanded periodontal evaluation implementing protein markers and DNA microbial probes for early disease detection. The oral epidemiology component is also working with the BLSA to increase minority enrollment thereby increasing the diversity of the BLSA population base. The implementation of these additional areas of investigation within the BLSA, present an opportunity to enhance the overall understanding of age related changes in the oral cavity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00570-02 EPI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Some Power Considerations When Deciding to Use Transformations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert	Chief Statistician	EODPP, NIDR
Zion, Gary	Computer Programmer/Analyst	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology and Oral Disease Prevention Program

SECTION

Analytical Studies and Decision Systems Branch

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.35	.35	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Conventional wisdom suggests that one either perform a power transformation (log, square root, etc) to data derived from studies with small sample sizes whose response variables have non-normal distributions before analysis, or use a distribution-free procedure such as a rank transformation or a randomization test procedure. To better appreciate the effect of specific alternatives on both the type I error and power of detecting differences between treatment groups, simulation studies were conducted assuming specific gamma distributions $G(r, \theta)$. A simple two group design was assumed. The reference group always had a average disease level $\mu = r \cdot \theta = 3.0$, and the treatment group always had means whose percentage reductions ranged from 0% to 50%. By varying the shape parameter r from 1, 2, 4, 8, 16 one could investigate distributional profiles having almost symmetric distributions (r = 16) to those with highly skewed distributions (r = 1 or 2). Six statistical test procedures were compared. All test procedures were robust relative to the type I error. The UMP test based on a ratio of sample means produced the greatest power for all combinations of n, r and R_T. The power losses associated with the randomization test, the t-test on original scale, and the t-test on the square root scale were very small, (3% to 6% in absolute value) for n = 10 and 15, and less than 2% for group sizes of 25 or more. The power loss associated with the t-test on the log scale was much larger, ranging from 5% to 10% smaller power than the t-test on original scale. The Wilcoxon rank test produced similar results to that of the LOG t-test for small samples. The loss in power for the unshifted LOG test could be recouped by use of a shifted LOG (x + c) test. The same procedures based on differences in sample means were then compared for comparable lognormal distributions. Here the log transformation performed the best, better than the Wilcoxon rank test, and both considerably better than the t-test on the original scale. These results suggest that statistical inferences can be highly dependent on both the distributional form of the response variable and the scale of measurement used in the statistical analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00582-01 EPI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 charactars or less. Title must fit on one line between the borders.)*

Current techniques for measuring dental fluorosis; Issues in data analysis

PRINCIPAL INVESTIGATOR *(List other professional parsonnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert Chief Statistician EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director, EODPp

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spaca provided.)*

The actual fluorosis prevalence value reported for a population can vary considerably among different scoring systems and within a scoring system due to intrinsic factors defining a case. Currently popular scoring systems evaluate the level of fluorosis are based on different measurement units, diverse numbers of sites per person, and distinctly different groupings of clinical symptoms. Intrinsic factors for a scoring system include the inclusion of a questionable category, together with both the level of fluorotic involvement and the number of sites within a subject required for case definition. None of these factors are related to the level of fluoride exposure in the examined population. Case definitions for each scoring system are desirable, essential for obtaining prevalence estimates, but currently not available for all scoring systems. Dean's scoring system has been the most widely used, includes a case definition, and thus, despite its peculiarities, will probably continue to play the role of reference standard. Ratios of fluorosis prevalence magnitudes, as evidenced by odds ratios, can be more stable between scoring systems for comparing groups having different fluoride exposure levels. There is a strong correlation among extent and specific measures of fluorosis severity for the DI and TSIF scoring systems, as well as within each scoring system separately. Parallel patterns in fluorosis severity were found among groups exposed to different levels of fluoride. The effects of fluoride exposure are best understood using relative measures contrasting severity levels of fluorosis for two or more fluoride exposure levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00583-01 EPI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The NIDR Amalgam Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert	Chief Statistician	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Albertini, Tullio	Special Asst for Program Mgmt & Prof Affairs	EODPP, NIDR
Zion, Gary	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director, EODPP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.40	.40	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In September of 1992 a collaboration between the NIDR and the Air Force Medical Corps was established to conduct a comprehensive study on the effects of mercury vapor from dental amalgams as a component of the Air Force Health Study (AFHS) of Vietnam Veterans. The surrogate measure used to assess exposure in this study is the total number of amalgam surface years for a study participant. Several health outcomes are being investigated as part of this study protocol. These include information on the neurological system, renal system, endocrine system, immunological system, and several psychological characteristics of study participants. The clinical examinations were completed by the end of March 1993. There was a 98% compliance rate for the NIDR amalgam study among potential AFHS participants. Blood and urine samples were collected from all participants as part of the study protocol and are being analyzed by Mayo Clinic's laboratory and validated by an expert reference laboratory. Lead blood assays were also performed for a 15% subsample of these participants. The 1992 clinical data is currently being processed and will be available to the NIDR shortly. Immediate attention will be given to a study involving the associations between Hg concentrations in urine and/or blood and dental amalgam exposure. A detailed analytical plan is currently under development for the main study involving the analysis of major health outcome variables. This protocol will be drafted by NIDR staff, reviewed and revised by senior Air Force Health Study investigators, and co-authorship of study protocol finalized in early fall on 1993.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00602-01 EPI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Missing Teeth in the Definition and Calculation of Caries Increments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kingman, Albert Chief Statistician EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Office of the Director, EODPP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

When defining caries increments in children and young adults it is customary to refer to DMFS increments as net increment, NI(DMFS), and it is defined as the number of sound surfaces which become "decayed" (SD+SF+SM) minus the number of "decayed" surfaces that become sound (DS+FS+MS). If increment is defined as the difference in time-specific DMFS scores, ie. DIF(DMFS) = DMFS2 - DMFS1, then it is easily shown that NI(DMFS) = DIF(DMFS).

Many investigators define increments using change in the DFS scores for older adult populations because the reason for missing teeth is often unknown. However, the consequence of ignoring missing teeth in the definition produces inconsistencies between increment estimates because missing surfaces behave both like sound and "decayed" surfaces. If one were to estimate DFS increment by NI(DFS), the analog of NI(DMFS), where NI(DFS) = (SD+SF)-(DS+FS), it would no longer be equivalent to the analog DIF(DFS), where DIF(DFS) = DFS2 - DFS1 as one might expect. But rather the relationship between increment estimates now becomes DIF(DFS) = NI(DFS) + ("new lesions")-("reversals"). The "new lesions" (MD and MF) and "reversals" (DM and FM) terms are a consequence of not accounting for missing surfaces. Even if the true cause of tooth loss could be determined for all missing teeth, neither increment estimate appropriately counts surfaces for which caries progresses, treated or untreated, to the point where an extraction is performed.

Research Projects

Molecular Epidemiology & Disease Indicators Branch

Epidemiology & Oral Disease Prevention Program

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00044-23 MSS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Handling of Microbial Strain Information by Computers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

McManus, Candace	Microbiologist	MSS, MEDIB, EODPP, NIDR
Krichevsky, Micah I.	Guest Researcher	MSS, MEDIB, EODPP, NIDR

COOPERATING UNITS *(if any)*

See attached page

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

Microbial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.27	0.27	1.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The MSS is developing a unified computer coding system for microbial information which is becoming an international standard for communicating strain data. The original bacterial system now includes the algae, yeasts, fungi, protozoa, and hybridomas.

Strain data are being entered into computers to provide: data on specific organisms; identification of unknown isolates; definition of parameters of taxa; aids in quality control of tests, methods, and laboratories; and communication of data via common format. Files of primary data on microorganisms found in the oral cavity and related types provide a resource for ecological and epidemiological dental research. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

The MSS analyzes the phenotypic data submitted by cooperating reference laboratories to the International Working Group on Mycobacterial Taxonomy in order to elucidate the taxonomic relationships within this genus. The latest analysis demonstrated at least one new distinct group of clinically important mycobacteria.

With EPA and ATCC staff, the MSS is building databases to aid in risk assessment of release of genetically engineered organisms in the environment, including features of microorganisms used in genetic manipulation and biotechnological processes and redefinition of taxonomic boundaries of such organisms.

This project's immediate goal of publication is to make a database of descriptions of all known bacterial species. No government facilities or resources are involved in this last goal.

Z01 DE00044-23

COOPERATING UNITS: E. Baron, Wadsworth VA Hospital
M. Grahn, Food and Drug Administration
C. McManus, Food and Drug Administration
S-C. Jong, American Type Culture Collection
L. Blaine, American Type Culture Collection
M. Segal, Environmental Protection Agency
L. Wayne, Long Beach VA Hospital
W.E.C. Moore, Virginia Polytechnic Institute and State University
L.V.H. Moore, Virginia Polytechnic Institute and State University
J. Holt, Bergey's Manual Trust
W. Hensyl, Williams & Wilkins, Publishers

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00250-16 MSS

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Algorithms for Microbial Systematics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walczak, Cynthia A.	Computer Scientist	MSS, MEDIB, EODPP, NIDR
Krichevsky, Micah I.	Guest Researcher	MSS, MEDIB, EODPP, NIDR
McManus, Candace	Microbiologist	MSS, MEDIB, EODPP, NIDR

COOPERATING UNITS *(if any)*

See attached page

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

Micobial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.51	0.51	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Microbial Information System (MICRO-IS) is an ongoing project to enter, retrieve, and analyze microbiological data for epidemiological, diagnostic, taxonomic, ecological, and regulatory uses. The long term goal is to establish a worldwide data network at a series of cooperating centers. A mainframe version of the MICRO-IS is currently used extensively for management and analysis of strain data by the MSS and also by the FDA and EPA their regulatory roles. The latest thrust of this effort is development of a portable version of the MICRO-IS for installation on a wide range of computers including personal computers, minicomputers, and mainframes. This version is now being distributed and accepted on a worldwide basis.

This project is now being done as a collaborative effort between the not-for-profit Bergey's Manual Trust, the not-for-profit Bionomics International, and Williams & Wilkins, Publishers, to adapt the programs for scholarly publication of descriptions of all known species of bacteria as both a database and in book form. The programs as such will continue to be freely distributed to the scientific community.

Z01 DE00250-16

COOPERATING UNITS: M. Grahn, Food and Drug Administration
L. Blaine, American Type Culture Collection
M. Segal, Environmental Protection Agency
W.E.C. Moore, Virginia Polytechnic Institute and State University
L.V.H. Moore, Virginia Polytechnic Institute and State University
J. Holt, Bergey's Manual Trust
W. Hensyl, Williams & Wilkins, Publishers

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00598-01 MEDIB

PERIOD COVERED

June 18, 1993 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

PCR Typing Procedure for Genetic Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ann Miller-Chisholm	Health Scientist Administrator	MEDIB NIDR
Dale B. Mirth	Health Scientist Administrator	MEDIB NIDR

COOPERATING UNITS *(if any)*

American Type Culture Collection, Rockville, Maryland

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.04	0.04	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This pilot study is being carried out to develop and test gene mapping strategies that can be utilized in future national surveys and clinical studies related to oral and systemic diseases with dental manifestations. Whole saliva samples collected in the Baltimore Longitudinal Study of Aging will be used. The average number of buccal cells present in a 1 mL sample will be determined. Genomic DNA will be isolated from the buccal cells and subjected to first stage PCR amplification. The first stage PCR product will be quantitated and the amplified samples will be PCR typed at 10 loci. Manual versus automated gel analysis systems will be investigated. The potential for multiplexing samples will be investigated. Replicate samples will be examined for the effects of long term storage.

Research Projects

Analytical Studies & Decision Systems Branch

Epidemiology & Oral Disease Prevention Program

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00420-08 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of National Survey of Oral Health in School Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brunelle, Janet A.	Statistician (Health)	EODPP, NIDR
Oldakowski, Richard J.	Chief, SPU, ASDSB	EODPP, NIDR
Mercer, Paula	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.40	0.05	0.35

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A national survey of the Oral Health of School Children was conducted during the 1986-87 school year. Approximately 41,000 children were examined by 13 trained and calibrated dental teams. When compared to a similar survey conducted in 1979-80, DMFS had declined 37%. Mean DMFS was lower in all regions of the country; however, there were still regional variations as before. Approximately 50% of the children aged 5-17 were caries free in their permanent dentition. A monograph on caries status, "Oral Health of U.S. Children, 1986-87," was published. Mean caries experience for primary teeth for children aged 5-9 years showed a 26% decline from the 1979-80 survey. The dfs was lower in every region except Region VII, with the greatest decline observed in Reg. I. Approximately 50% of the children had no dfs; 28% had more than 4 surfaces d or f. Only 7.6% of the children aged 5-17 had sealants present. The average number of sealants in children with sealants was 4.2 per child. Microbiological samples of mutans streptococci and Lactobacillus were evaluated in relation to dental caries. Children with mutans counts of zero CFU/ml of salivary rinse had less than half as many DMFS as those with any detectable mutans. The level of caries rose with increases in mutans.

An estimate of the prevalence of dental fluorosis was made using Dean's Index on 2nd through 12th graders. 22% of children showed definite signs of fluorosis, 17% very mild, 4% mild, 1% moderate and 0.3% severe. Prevalence was about 5% less in the older age groups. Regional differences existed with respect to both prevalence and severity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00475-06 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Utilization, Treatment Needs, Cost, and Dental Disease in Veterans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Director	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Zion, Gary R.	Computer Programmer	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

Veterans Administration Outpatient Clinic, Tufts University, and Harvard University, Boston, Massachusetts

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.47	0.46	0.01

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Veterans Administration in 1963 initiated an interdisciplinary and longitudinal investigation of the normal aging process. Participants consisted of 2,400 men with stable living and work conditions in the Boston, Massachusetts area. From this panel, 1221 self-selected subjects between the ages of 25 and 75 volunteered for the Dental Longitudinal Study in 1968. These persons have received a complete dental examination every three years since 1968. The triennial examinations include a radiographic survey and a comprehensive clinical examination documenting dental caries, periodontal status, missing teeth, and oral hygiene.

This project is supplementing these clinical data with detailed utilization data from the dental offices visited by the panel members over the past ten years. The data collection is complete and the information has been integrated with the clinical data. The full dataset is being used as a source of previously unavailable longitudinal information as well as cross sectional information. Findings on episodes of use and nonuse of dental services were presented at the AADR. Manuscript reporting methods and results of study is in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00496-05 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determinants of Permanent Tooth Loss in Connecticut and North Carolina

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Winn, Deborah	Chief, ASDSB	EODPP, NIDR
Albertini, Tullio F.	Sp Asst for Prog Mgt	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASDSB	EODPP, NIDR

COOPERATING UNITS *(if any)*

University of Connecticut, Farmington, Connecticut

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.63	0.43	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this study are to measure permanent tooth loss and the factors which influence it. The study will be conducted in two (2) phases. The first phase will be independent of the second and will be a complete study without the second phase. Specific aims of Phase I are to describe 1) the biological condition of extracted teeth, 2) the sociodemographic, attitudinal, economic, and dental care-seeking characteristics of individuals who have extractions, and 3) selected characteristics of the dental providers who perform the extractions. Phase II will be conducted after the first phase and will collect information on patients whose teeth were treated with dental services that are alternatives to extraction for given biological conditions. These teeth will be controls for the extracted teeth and will allow the estimation of a model which explains the factors which influence the choice between extraction and its alternatives. The same practices will be used for both phases. Data from both Phases will be used to develop a more complete explanation of the relative significance of these factors for tooth loss.

The final sample totals 82 private practitioners (not including dentists in the nine clinics) in Connecticut and 68 dentists in North Carolina. Phase I field operations are nearing completion. Data have been collected from 72 dentists (and their patients) in Connecticut and from 60 dentists (and their patients) in North Carolina. In addition, almost all extracted teeth from patients of 60 dentists in North Carolina and 71 dentists in Connecticut have been assessed for caries and periodontal status. Preliminary inspection of the data reveal root caries to be a much greater cause of tooth extraction than previously expected. Finally, fifteen dentists have been recruited in Connecticut to assess the in-vivo periodontal status (pocket depth, attachment loss) of teeth that are subsequently extracted and examined in-vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00497-05 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Forecasting Dental Health and Utilization Using A Microsimulation Model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Director	EODPP, NIDR
Zion, Gary R.	Computer Programmer	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR
Winn, Deborah	Chief, ASDSB	EODPP, NIDR
White, Benjamin A.	Sr. Dental Research Investigator	EODPP, NIDR

COOPERATING UNITS *(if any)*

Cornell University, Department of Sociology, Ithica, New York and University of Michigan, Ann Arbor, Michigan

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.41	0.40	0.01

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A computer model which will generate condition forecasts of future tooth loss, dental status, service utilization and expenditures for individuals and families in the U.S. is being developed. These forecasts will be developed in considerable sociodemographic details. Dr. Caldwell, a microsimulation specialist at Cornell University, is principal investigator on the contract. Dr. Stephen Eklund of the University of Michigan is assisting in the development of the oral disease and conditions portion of the model. Several noted dental specialists and modeling experts are consultants to the project. Development of the model and the production of initial forecasts has been completed. Programming and testing of the sociodemographic and dental portions of the model are complete. Tests of the full model are being conducted by EODPP staff. Microsimulation is the approach being used. Starting from a representative sample of persons and families, the NIDR micro model will forecast tooth loss, dental health conditions, and dental service use for persons identified by age, gender, race, education, income, and other putatively important explanatory variables. Policy experiments with the full model are planned both for past times and also for future times. As a framework for synthesizing research findings, the NIDR micro model will provide a vehicle for carrying out experiments in which the latest dental research can be applied consistently and systematically to key dental policy issues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00515-04 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Periodontal Health in Adult Americans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kaste, Linda M.	Senior Staff Fellow	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.31	0.26	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The periodontal health for the Senior portion of the NIDR National Survey of Oral Health in U.S. Employed Adults and Seniors is being extensively studied. An analysis of the association between sociodemographic variables and periodontal status for Seniors 65 years and over, is being completed using SUDAAN. SUDAAN, 'SUrvey DAta ANalysis for multi-stage sample designs' has recently been brought to the lab. Analyses are being conducted on both the IBM mainframe and the new RISC/6000 Scientific Workstation to test the adaptation of the statistical software on the workstation for actual data. The previous concentration on periodontal disease from the survey has been directed at the Employed Adult portion. Outcome measures being analyzed include: loss of attachment, periodontal pocketing, gingival recession, oral hygiene, gingivitis, and presence of calculus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00517-04 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Study of Alveolar Bone Loss and Aging Among Healthy U.S. Males

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson Director EODPP, NIDR

COOPERATING UNITS *(if any)*

Veterans Administration and Tufts University, Boston, Massachusetts

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project is a cross-sectional and longitudinal analysis of alveolar bone loss (ABL) in aging men. Study subjects are 700 adult male participants in the VA Dental Longitudinal Study (VADLS). Bone loss is determined using existing intraoral periapical radiographs.

The VA-DLS has the most extensive longitudinal radiographic data base available with which to examine the factors associated with progressive alveolar bone loss. The existence of sequential radiographs permitted longitudinal analysis of the actual ABL experienced over a twenty year period at three year intervals. Full-mouth series of intra-oral periapical radiographs, obtained at three-year intervals, were computer digitized and used to measure ABL over time. Work is continuing on digitizing radiographs. Interim results have been presented at three meetings.

Percent remaining bone was measured at all interproximal sites at each of six time points. Over 87% of subjects had at least one site that had experienced a rate of ABL ≥10% over 15 years. 47% of subjects had more than 5 sites and approximately 20% had more than 11 sites with a rate of ABL ≥10% over the 15 years. Distribution of sites having more severe ABL was similarly skewed, with a relatively small number of subjects accounting for the majority of sites with severe ABL. The project will yield much improved estimates of the rate of ABL condition on putatively important explanatory variables.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00540-03 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tobacco Use and Oral Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Swango, Philip	Chief, FSS	EODPP, NIDR
Winn, Deborah M.	Chief, ASDSB	EODPP, NIDR

COOPERATING UNITS *(if any)*

Gary A. Giovino, Acting Chief, Epidemiology, OSH, CDC, Atlanta, Georgia

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section and Field Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.14	0.08	0.06

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Epidemiologic evidence has been accumulating that cigarette smoking is causally related to cancers of the larynx, oral cavity, and esophagus in both men and women. The mortality ratios for these cancers are similar for smokers of cigarettes, pipes, or cigars. A strong dose-response relationship exists. Compared with those who continue smoking, the risk of cancer decreases for those who quit smoking. Alcohol consumption is also an important risk factor for oral, pharyngeal, laryngeal, and esophageal cancer. The combination of smoking and alcohol acts synergistically to increase risk of these cancers.

There is mounting concern about the oral health consequences of the recent resurgence of smokeless tobacco use among teenage boys in the United States. While the evidence is strongest that smokeless tobacco causes cancer of the oral cavity, there is also evidence that the use of smokeless tobacco increases the risk of cancer of the pharynx, larynx, and esophagus. Smokeless tobacco also causes a variety of noncancerous and precancerous oral conditions, the most important of which is oral leukoplakia (other less serious oral conditions associated with the use of smokeless tobacco include gum recession and tooth loss).

The purpose of this study is to re-examine the relationships between tobacco use (cigarette smoking and smokeless tobacco) and oral health using recent large national data sets and examine the possible physiologic mechanisms through which tobacco acts on oral tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00542-03 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tooth Loss Among the Elderly in the United States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Kaste, Linda M.	Senior Staff Fellow	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Winn, Deborah	Chief, ASDSB	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.42	0.41	0.01

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this study are to describe tooth loss among the elderly population in the United States and how tooth loss varied among subgroups of the elderly population.

Analysis of tooth loss in the elderly population have been carried out using the Oral Health of U.S. Adults Survey, 1985-86. Findings suggest that while progress has been made in reducing the overall prevalence of edentulousness, there remains subgroups of the elderly population (poor, lower educated, lower income, black) that have significant rates of tooth loss.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00544-03 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Periodontal Health in Adolescent Americans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brunelle, Janet A.	Statistician	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASDSB	EODPP, NIDR

COOPERATING UNITS *(if any)*

Westat, Inc.; University of Minnesota; University of Tennessee; SUNY, Buffalo; Columbia University; VPI; Medical College of Virginia

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.56	0.46	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A study of the prevalence of early onset periodontitis in U.S. children aged 14 to 17 years has been completed and was published in October, 1991. In a national survey, the total number of adolescents affected by LJP was about 70,000. Seventeen thousand were estimated to have GJP and another 212,000 adolescents had incidental LA (≥3mm on 1 or more teeth). Blacks were at greater risk of all forms of early onset periodontitis than whites. Males were more likely (4.3 to 1) to have GJP than females when other variables were statistically controlled.

A major contract to relocate, re-examine and collect risk factor information on these children began in October, 1991. Research objectives are to: a) assess the progression of periodontal destruction among the cases of early onset periodontitis, b) characterize the microbial ecology of the sub-gingival plaque among persons with early onset periodontitis, and c) compare the presence and concentration of selected putative pathogens and high-resistance factors among individuals with early onset periodontitis to controls.

A sample of all cases whose oral examinations from the 1986-87 survey indicated early onset periodontitis or other severe periodontal problems was selected for study. Two controls per case matched by age, gender, race and geographic location were also located and invited to participate in the study. During FY'93, examinations were made on approximately 265 young people 19-25 years of age. Full mouth oral examinations for periodontal measures and dental caries were conducted. Biological specimens, blood, gingival crevicular fluid and subgingival plaque fluid were also collected. A questionnaire including such variables as medical history, family history, and dental utilization was administered at the time of the oral exam. Laboratory analyses of biological specimens is under way. Dental examination data is being edited and prepared for analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00545-03 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Longitudinal Studies of Periodontal Health in Norwegians and Sri Lankans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Director	EODPP, NIDR
Löe, Harald	Director	NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR

COOPERATING UNITS *(if any)*

Morrison, Edith	Univ of Texas Health Center

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.09	0.07	.02

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analysis of the progression of periodontal conditions from longitudinal data on subjects from Norway and Sri Lanka are ongoing. Recession and tooth loss due to the progression of periodontal destruction were analyzed. Evaluation of gingivitis status and its relation to progression of periodontal disease is underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00565-02 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparison of Mutans Counts from Three Selective Media

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brunelle, Janet A.	Statistician (Health)	EODPP, NIDR
Little, Wayne A.	Microbiologist	PIRS, OD, NIDR

COOPERATING UNITS *(if any)*

Horace M. Stiles, Health Science Administrator, DRG, NIH

LAB/BRANCH

Analytical Studies and Decisions Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20897

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.06	0.06	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over two thousand salivary rinse samples were collected during the 1986-87 national survey of U.S. schoolchildren. Specimens collected with this technique were plated on three selective media: MSB, GSTB and TSY20B. Counts of mutans streptococci were determined on all three media. Mean DMFS were computed for all children aged 7 to 17 who had rinse samples. 1109 specimens had readable plate counts for mutans on all three media. In over one-fifth of the samples from each media, no CFU of mutans were detected.

Mean mutans counts (CFU/ml of salivary rinse) were approximately 1.5×10^4 for MSB, 1.2×10^4 for TSY20B and 1.7×10^4 for GSTB. Repeated measures ANOVA indicated significant differences ($p<.0001$) between counts on different media. However, correlations between media were high and differences were normally in the same direction with GSTB highest and TSY20B lowest.

Associations with dental caries were measured for all three media. Mean CFU for those with zero DMFS were 30 to 40% lower than for children with 1 or more DMFS. Mean DMFS scores were 50% lower for groups with zero mutans counts versus those with any mutans for all three media. (approximately 2 DMFS for 0 mutans counts and 4 DMFS for those with mutans). Odds ratios for the association between mutans and caries were approximately 2.2 for all three media, i.e. children with mutans were about twice as likely to have caries as those with no mutans ($p<.0001$).

Counts from all three media were able to differentiate between groups of children with or without dental caries reflecting the strong association between dental caries and mutans and making the salivary rinse a viable collection technique.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00568-02 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Cost Curve for Community Water Fluoridation Based on Water Usage Information

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brown, L. Jackson	Director	EODPP, NIDR
Zion, Gary R.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

Florida Health Department, Tallahassee, Florida

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.26	0.16	0.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous data relating to the cost of water fluoridation have frequently been limited to variable costs such as chemicals and other materials. The purpose of this study was to measure opportunity costs and to estimate a cost curve for water fluoridation. Data were collected from 44 Florida communities which had initiated community water fluoridation between 1981 and 1989. Equipment installation and engineering costs were derived from actual invoices and adjusted to 1988 dollars. Output of the water systems was measured by water usage and community population.

For large systems, average costs approached an asymptote at $0.21 per person per year when population was used as a measure of output and $1,199 per year to fluoridate one million gallons of water daily. Most of the economies of scale were exhausted with water systems serving moderate sized towns with 10,000 to 50,000 people. While water usage is conceptually the preferred output measure, results indicate that population can serve as a very good proxy when water usage is not available. L shaped average cost curves were very good models of fluoridation. Long range average cost curves exhibited economies of scale.

Economic considerations which implied nonlinear constraints on the model parameters were analyzed. These constraints are nearly satisfied by the linear model and had a very small effect on the parameter estimates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00572-01 ASDSB

PERIOD COVERED

April 1, 1993 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cost and Utilization of Dental Services - National Medical Expenditure Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

White, Benjamin (Co-Principal Investigator)	Senior Dental Research Investigator	EODPP, NIDR
Marcus, Stephen (Co-Principal Investigator)	Senior Epidemiologist	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Robinson, Dina Larach	Health Promotion Research Specialist	EODPP, NIDR
Oldakowski, Richard	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Decision Systems Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.57	.47	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In 1987, the National Medical Expenditure Survey (NMES) II was conducted by the Center for General Health Services Intramural Research, Agency for Health Care Policy and Research. The survey provides extensive information on health expenditures by or on behalf of American families and individuals, the financing of these expenditures, and each person's use of services during the period from January 1 to December 31, 1987. The NMES II Household Survey is based on a national probability sample of the civilian, noninstitutionalized population living in the community. The sample is designed to provide a larger representation of population groups of special policy interest to the Federal Government than would have been obtained from a random sample. These groups include poor and low income families, the elderly, the functionally impaired, and black and Hispanic minorities.

As part of NMES II, information was collected on the type of dental services provided, total expense, and sources of payment for all dental services. This project will use the data to examine several issues related to dental services and oral epidemiology, such as: 1) overall dental utilization and expenditure patterns; 2) geographic variation in practice patterns for dental services; 3) demographic and socioeconomic variation is use of dental services; 4) associations between the use of dental services and other health care services; 5) relationship between the use of dental services and reported oral and general health status; 6) use of dental services by Native Americans and Alaska Natives; 7) use of dental services and health related behaviors, including care seeking and preventive care; 8) usual source of medical and dental care and reasons for lack of a usual source of dental care; and 9) health insurance status and use of dental services.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00573-01 ASDSB

PERIOD COVERED

April 1, 1993 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Patterns of Care, Outcomes, and Cost of Oral Cavity and Pharyngeal Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

White, Benjamin A.	Senior Dental Research Investigator	ASDSB, EODPP, NIDR
Winn, Deborah	Chief, Analytical Studies and Decision Systems Branch	EODPP, NIDR
Kleinman, Dushanka	Deputy Director	NIDR
Marcus, Stephen	Senior Epidemiologist	ASDSB, EODPP, NIDR
Oldakowski, Richard	Computer Programmer	ASDSB, EODPP, NIDR

COOPERATING UNITS *(if any)*

Applied Research Branch, Surveillance Program, Division of Cancer Prevention and Control, National Cancer Institute and the Division of Beneficiary Studies, Office of Research, Health Care Financing Administration

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Decision Systems Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.37	.37	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

EODPP has been working with the National Cancer Institute (NCI) to obtain the SEER/Medicare Linkage project database for use in a number of investigations in oral cancer. The Linkage Project is a collaborative effort by the NCI and the Health Care Financing Administration (HCFA) to link the NCI's Surveillance, Epidemiology, and End Results (SEER) Program data base, which contains information on cancer cases diagnosed and reported in nine geographically distinct population-based tumor registries, and HCFA's Medicare statistical system (MSS), which contains extensive billing information for the health care of the disabled and more than 95 percent of the elderly.

Initial work has begun at NIDR on developing an analysis plan for oral cavity and pharyngeal cancer utilizing this linked data base. The objectives of this study are to: 1) identify regional (SEER) variations in the choice of first-course of cancer directed therapy among individuals 65 years of age and over with oral cavity and pharyngeal cancer, by site of cancer; 2) add to the information currently available concerning the effectiveness of alternative therapies used to manage oral cavity and pharyngeal cancer among the elderly population; and 3) estimate the lifetime costs associated with oral cavity and pharyngeal cancer. For the years 1984 to 1989, 7,014 incident cases of oral cavity and pharyngeal cancer are contained in the data base, representing over 32,400 person-years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00579-01 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dental Caries and Selected Microbiological Determinations in Hispanic Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Brunelle, Janet A.	Statistician (Health)	EODPP, NIDR
Mercer, Paula	Computer Programmer	EODPP, NIDR
Oldakowski, Richard	Chief, SPU, ASDSB	EODPP, NIDR

COOPERATING UNITS *(if any)*

Stanley B. Heifetz	University of Southern California
Jorgen Slots	University of Southern California

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.20	0.15	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A sample of approximately 200 children of Hispanic ancestry who are participating in a sealant project in East Los Angeles are being clinically and microbiologically examined. Questionnaires to check residency and heritage information are obtained. Participants are in grades two and three.

A dental examiner, screening minority children for larger sealant study, is examining deciduous and permanent molars for dental caries according to NIDR criteria. Microbiological specimens are collected using the salivary rinse system designed for NIDR National Survey of Children.

Determination of mutans streptococci and lactobacillus counts are being made in the laboratory at the University of Southern California. Analyses of relationships between dental caries status and microbiological counts are under way.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00580-01 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Occupation and Reproductive Health of Women Dentists

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kaste, Linda M. Senior Staff Fellow EODPP, NIDR

COOPERATING UNITS *(if any)*

American Dental Association, University of North Carolina, and NIEHS

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.50	.50	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A national survey of occupational exposures assessing the relationships of the practice of dentistry and the reproductive health of women dentists is being conducted via a mailed questionnaire. Approximately 5,500 women who graduated from dental school from 1977 to 1986 and were 31-40 in the spring of 1992 were mailed questionnaires. The sample was chosen to allow for the opportunity to have dental occupational exposures during reproductive ages. The methodology builds upon work conducted at NIEHS on occupational exposures focusing on amalgams and nitrous oxide and primarily the reproductive outcomes of time to pregnancy and spontaneous abortion. This is the first attempt for the acquisition of these types of data via self administered questionnaire. An initial mailing, post-card follow-up and thank you, second questionnaire, and a follow-up letter from the American Dental Association, for a total of four mail contacts made during this fiscal year. A response rate near 60 percent has been achieved and telephone solicitation has begun to increase the response rate. A poster presentation was made at the 1993 IADR/AADR meeting on information from the pilot study of the project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00581-01 ASDSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biomarkers for Oral Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Winn, Deborah M.	Chief, ASDSB	EODPP, NIDR
Robbins, Keith C.	Chief, LCDO	IP, NIDR
Schwartz, Joel L.	Section Chief, BDMS, MEDIB	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.21	0.06	0.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Twelve thousand new cases of oral cancer occur annually in the United States. Recent laboratory developments have identified biomarkers measurable in human fluids or tissues which may help further our understanding of the process of carcinogenesis and identify persons at especially high risk of oral cancer. Among these are p53 tumor suppressor genes and heat shock proteins. Evidence from examination of dietary factors in oral cancer cases and controls, from studies of other cancers, from laboratory studies, and from some very recent chemotherapeutic trials also suggests the importance of nutritional factors in cancer etiology. This project will examine biomarkers and nutritional status in a cohort of persons with oral premalignant lesions who are monitored for changes in biomarker status or the development of cancer. Data collection activities and some laboratory assessments will be managed under contract. If the research can identify biomarkers which precede disease in a predictable way, there may be the potential to prevent oral cancer, screen for more successfully, and/or treat it at an earlier stage.

Research Projects

Health Assessment Branch

Epidemiology & Oral Disease Prevention Program

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00464-06 HAB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural History of Oral Manifestations of HIV Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Philip A. Swango	FSS, HAB, EODPP, NIDR
Dushanka V. Kleinman	OD, NIDR
Philip Fox	CIPCB, IRP, NIDR
Ruth Nowjack-Raymer	DPS, DPHPB, EODPP, NIDR
Carla Bock	FSS, HAB, EODPP, NIDR
Linda Kaste	AES, ASDSB, EODPP, NIDR

COOPERATING UNITS *(if any)*

Walter Reed Army Institute of Research

LAB/BRANCH

HAB, EODPP, NIDR

SECTION

Field Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.65	1.0	0.65

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

United States military personnel and dependents who have tested seropositive for the Human Immunodeficiency Virus (HIV) are given medical examinations and treatment at Walter Reed Army Medical Center. Subjects are also invited to participate in a research protocol to study the natural history of HIV infection, conducted by the Walter Reed Army Institute of Research. An oral health research component conducted by NIDR is a part of this natural history study.

The oral component documents the prevalence and incidence of oral pathologic conditions in relation to the stage of HIV infection and systemic disease. Risk factors associated with these conditions are also characterized, and the role of oral manifestations as early predictors or markers of disease progression are studied. Areas of emphasis are mucosal diseases, periodontal conditions, candidal infections, and salivary constituents. Results to date show an increase in mucosal lesions as T4 cell counts decrease with progression of HIV disease. The most commonly observed mucosal lesions were oral candidiasis and hairy leukoplakia. Destructive periodontal disease has occurred in about 25 percent of subjects examined. Longitudinal studies showed that about 30% of persons free from mucosal diseases at entry presented with oral lesions after six months of follow-up.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00501-04 SSDM

PERIOD COVERED

October 1, 1992 to December 31, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Surface-Specific Attack Patterns In Primary Teeth From Two National Surveys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Li, Shou-Hua	Statistician (Health)	NIDR
Others:			
	Kingman, Albert	Chief Statistician	OD, NIDR
	Swango, Philip	Chief, Field Studies	FS, EODPP, NIDR
	Webb, Kimberly	Statistical Assistant	SSDM, EODPP, NIDR

COOPERATING UNITS *(if any)*

Ronald Forthofer, Private Consultant

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1		0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study was to investigate whether changes had occurred in attack rates in primary teeth during the 1980's. Data on decayed and filled surfaces (df) in 5-9-year old children from the 1979-80 and 1986-87 NIDR dental caries surveys were analyzed. The attack rate was defined as the number of decayed and filled surfaces per 1000 surfaces at risk.

The prevalence of df surfaces declined from an overall mean of 5.31 in 1979-80 to 3.91 in 1986-87. Surface-specific caries attack rates were found to be similar in both surveys. The rank order of the largest six attack rates were: occlusal surfaces of 2nd and 1st molars, distal surface of 1st molar, mesial of 2nd molar, lingual of upper 2nd molar and buccal of lower 2nd molars. The tooth-specific attack rates were, from high to low: 2nd molar, 1st molar, central incisor, lateral incisor and cuspid.

There was no appreciable difference reduction in caries attack rates between the occlusal and proximal surfaces of primary teeth from the 1980 to 1987 NIDR survey. This was in contrast to greater reductions in caries attack rates reported in proximal surfaces of permanent teeth between the 1971-74 HANES survey and the 1980 NIDR survey and also permanent teeth between 1980 to 1987 survey.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00503-04 SSDM

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Documentation of Public Use Files--1986-87 and 1979-80 Surveys of School Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Snowden, Cecelia B.	Chief, Samp., Stat., & Data Mgmt.	SSDM, EODPP, NIDR
Others:	Davis, Trenita	Statistical Assistant	SSDM, EODPP, NIDR
	Lee, James	Computer Assistant	SSDM, EODPP, NIDR
	Webb, Kimberly	Statistical Assistant	SSDM, EODPP, NIDR

COOPERATING UNITS *(if any)*

Westat, Inc.
Rockville, Maryland

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During 1986-87 the National Survey of Oral Health in School Children was conducted by Westat Inc. in cooperation with NIDR to monitor the prevalence of oral diseases in school children, grades K-12, throughout the contiguous United States and Hawaii. The 1986-87 survey was a replication and extension of the NIDR National Dental Caries Prevalence Survey conducted in 1979-80, also by Westat, which established baseline estimates on the prevalence of dental caries, gingivitis and dental restorative treatment needs. Both surveys utilized multi-stage probability samples of over 39,000 school children enrolled in grades K-12 to represent over 43 million children enrolled in public or private schools in the seven geographic regions of the U.S. In the 1986-87 survey, additional assessments were made for dental fluorosis, soft tissue lesions, and the use of smokeless tobacco. Residential histories, health and demographic data were collected for each child participating in the clinical examination.

The objective of this continuing collaborative effort is to document the survey designs and produce public use tapes for the 1979-80 survey, and to provide clinical protocols and statistical methodologies for calculating national or regional estimates, weights and sampling errors. The documented tapes generated by this effort were released for public use by the National Archives.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00504-04 SSDM

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Analysis of the 1986-87 and 1979-80 NIDR Surveys of School Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Snowden, Cecelia B. Chief, Samp., Stat,, & Data Mgmt SSDM, EODPP, NIDR

COOPERATING UNITS *(if any)*

Westat Inc.
Rockville, Maryland

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During 1986-87 the National Survey of Oral Health in School Children was conducted by Westat Inc. in cooperation with NIDR to monitor the prevalence of oral diseases in school children, grades K-12, throughout the contiguous United States and Hawaii. The 1986-87 survey was a replication and extension of the NIDR National Dental Caries Prevalence Survey conducted in 1979-80, also by Westat, which established baseline estimates on the prevalence of dental caries, gingivitis and dental restorative treatment needs. Both surveys utilized multi-stage probability samples of over 39,000 school children enrolled in grades K-12 to represent over 43 million children enrolled in public or private schools in the seven geographic regions of the U.S. In the 1986-87 survey, additional assessments were made for dental fluorosis, soft tissue lesions, and the use of smokeless tobacco. Residential histories, health and demographic data were collected for each child participating in the clinical examination.

The objective of this ongoing collaborative effort is to develop generalized variance models for selected non-binary statistics common to both surveys, to calculate correlations between the two surveys and to measure design effects in order to establish optimal cluster sizes for future national surveys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00527-03 SSDM

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Software for Analyzing Data From Complex Dental Surveys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Snowden, Cecelia B. Chief, Samp., Stat., & Data Mgmt SSDM, EODPP, NIDR

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Center for Disease Control
Hyattsville, Maryland

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of a data analysis software package (SUDAAN) that is appropriate for the analysis of complex sample surveys is an ongoing project of the National Center for Health Statistics through a contract with Research Triangle Institute (RTI). SUDAAN is a collection of several high level statistical procedures that employ state-of-the-art methodology, Taylor series or Delta method of estimation for analyzing data from complex sample survey designs. Data from two Children's, Adults and Seniors Dental Surveys are being used to analytically test and evaluate SUDAAN in five areas:

(1) appropriate methodology for complex survey samples
(2) portability
(3) reliability/numerical accuracy
(4) computational efficiency
(5) ease of modification/enhancement

Work is proceeding in these areas with SUDAAN on the mainframe.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00528-03 SSDM

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods and Software for Analysis of Geographic Referenced Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Snowden, Cecelia B. Chief, Samp., Stat., & Data Mgmt SSDM, EODPP, NIDR

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Centers for Disease Control
Hyattsville, Maryland

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is an ongoing research and development project that includes automated cartography, geographic information systems, and related statistical methods and software, that will generally and/or specifically be used to meet NIDR data analysis goals in studying minority dental health status and other studies such as tooth loss. Included in this project is an analysis and documentation of existing technology and application of new statistical methods and technology to NIDR data sources.

Research Projects

Disease Prevention & Health Promotion Branch

Epidemiology & Oral Disease Prevention Program

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00577-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prevention of Dental Caries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Selwitz, Robert H.	Dental Epidemiologist	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Gift, Helen	Chief, DPHP Branch	EODPP, NIDR
Cherry-Peppers, Gail	Post-Doctoral Fellow	EODPP, NIDR
Mercer, Paula	Computer Specialist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.60	.55	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prevention of dental caries is a key focus of research activities of staff. Both fluorides and dental sealants are topics of research investigation and science transfer. Research activities have been undertaken by staff regarding the use of fluoride and dental sealants including publications on the use of fluoride products by children under the age of two years, a vital and health statistic monographs and three journal articles on caries preventive behaviors in the U.S. and an analysis of community based sealant programs.

Even when dental caries prevalence has declined markedly in population groups exposed to beneficial levels of fluoride, decay in the pits and fissures of teeth persists as a problem. Such was the case in rural Nelson County, Virginia, where after 11 years of a school-based program of self-applied fluoride therapy, 92 percent of the remaining decay was confined to the pits and fissures. Final examinations for dental caries experience (DMFS index) were conducted in the fall of 1983. At this time, a dental sealant program was added to the ongoing fluoride program. Children ages 6-7 and 12-13 were eligible to receive pit and fissure sealants on their teeth. Dental caries data from the fall examination served as baseline information for those children who participated in the sealant phase of the study. In succeeding years, new groups of 6- and 12-year-olds were enrolled in the project. Treatments continued for four years. Final examinations were conducted in September 1987. After four years, the overall mean caries score for 416 children who received dental sealants and fluorides was 51 percent lower than the comparable score for 762 children (the same ages four years earlier in 1983) who received fluoride therapy only. A manuscript describing findings for dental caries and retention of dental sealants has been submitted for publication. Based on extensive literature review and analytic projects described above, staff are designing a cost effectiveness study of dental sealants to be begun next year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00578-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Fluoride Accumulation and Effects in the Body

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Selwitz, Robert H.	Research Dentist	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR
Kingman, Albert	Chief Statistician	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Brunelle, Janet A.	Statistician (Health)	EODPP, NIDR
Kaste, Linda M.	Senior Staff Fellow	EODPP, NIDR
Mirth, Dale B.	Health Scientist Administrator	EODPP, NIDR
Streckfus, Charles F.	Senior Staff Fellow	EODPP, NIDR

COOPERATING UNITS *(if any)*

University of Connecticut, Medical College of Georgia, University of Iowa
University of Michigan, University of North Carolina at Chapel Hill, and NCHS

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.96	.81	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fluoride accumulation and bodily effects and inter-relations between dental caries, dental fluorosis, and the use of fluoride receive high priority in the DPHPB. Clinical studies undertaken involve the assessment of dental fluorosis in children exposed to differing sources and levels of fluoride. The first study is a follow-up survey of children in Illinois and Nebraska, who have been exposed to different levels of fluoride in their drinking water. Each child was examined for dental caries and dental fluorosis using standard indices. Data from all study sites have undergone preliminary analyses, and investigators are developing an initial manuscript focusing on descriptive findings for the Illinois communities. The second study, a follow-up to a clinical trial on effective fluoride treatments conducted in Springfield, OH, was conducted to determine the prevalence of fluorosis in each of the three experimental groups two years post-treatment. Data from this examination have been analyzed and a report is being prepared for publication. DPHPB staff coordinated an NIDR sponsored workshop on methods for assessing fluoride accumulation and its effects in the body in January 1993. The goal of the 2½ day workshop was to review available scientific information regarding fluoride accumulation and bodily effects and to develop a comprehensive research agenda to address workshop objectives. The agenda included formal presentations, discussion, working group sessions, and reports of working group deliberations. Plans have been made to publish the proceedings of the workshop in a scientific journal. Research activities undertaken by EODPP staff regarding the appropriate use of fluoride papers on the use of fluoride products by children under the age of two years for presentation at the 1992 annual session of the AAPHD and publication in the Journal of Public Health Dentistry (in press). In addition, several lectures on the appropriate use of fluorides were prepared and delivered at dental schools and meetings of public health workers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00584-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Minority Oral Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robinson, Dina	Health Promotion Research Specialist	EODPP, NIDR
Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Cherry-Peppers, G.	Post Doctoral Fellow	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Kaste, Linda	Staff Fellow	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.19	1.09	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Improving oral health in the U.S. requires addressing high risk individuals many of whom are minorities. An extensive literature review to establish the basis for a community demonstration research project in a minority population has been conducted. A study design has been developed and is being evaluated for implementation. Staff have also developed a summary of oral health status of Black adults.

Staff are analyzing NIDR and NCHS data with a specific focus on oral health behaviors and oral health status of Black Americans. These analyses have resulted in presentations at professional meetings and manuscripts for publications.

Staff from the Health Promotion Section, in collaboration with a staff member form the Analytical Studies and Decisions Systems Branch, are developing an issues paper dealing with Hispanic Oral Health in the United States with special focus on access to care and available clinical disease indicators. This manuscript along a more extensive outline of Hispanic oral health issues and methodological considerations is being developed to guide the deliberations and discussions of a working group that will be convened during Fall 1993 to address Hispanic oral health issues and develop a proposed research agenda.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00585-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prevention of Oral Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Oldakowski, Richard	Computer Specialist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.12	.12	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oral soft tissue lesions, precancers and cancers are among the most serious, and in the case of oral cancer life-threatening, oral conditions. The risk factors for many of these conditions are clearly identified as comorbidities with medical conditions and treatments and with high risk behaviors such as tobacco and alcohol use. To that end, these conditions and the risks leading to them are clear targets for health promotion. The Branch is actively pursuing research which identifies correlates of knowledge, opinions and practices associated with these oral conditions and brings focus to intervention research which could improve strategies to reduce the incidence and prevalence of these conditions and associated risk behaviors. Staff have been conducting analyses of the 1990 NHIS data regarding knowledge of the risks, signs, and symptoms of oral cancer. These analyses were the basis of a presentation at the 1993 IADR session and are being expanded for a manuscript for publication. Staff are collaborating with a group of researchers at the University of Maryland regarding a statewide study of the knowledge, opinions and practices of the public and health care providers regarding oral cancer. Staff have been active in interagency forums on oral cancer and associated risk behaviors which have been held to identify research and program needs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00586-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Women's Oral Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Redford, Maryann	Public Health Specialist	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Snowden, Cecelia	Section Chief	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.59	.49	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Women's health has become a major priority for research at NIH. Branch staff have participated with other NIH scientists in developing clinical trial and community demonstration research agendas for women's health. Extensive literature reviews have been conducted to assess the interaction of oral health with systemic health and to determine how oral health fits within the contexts of these larger research initiatives. For instance, evaluations of the utility of oral biomarkers for systemic diseases and conditions have begun with a focus on their value in large scale epidemiologic studies.

Demographic, general health, economic, social, and behavioral trends identified by NIH expert working groups as concerns for the upcoming decade have provided impetus to re-examine women's oral health within this broad context. Encouraged by the enthusiastic response to an IADR session in 1992 which focused on potential issues surrounding women's oral health, Branch staff organized a women's health symposium for the 1993 meeting. One staff member presented an overview of women's oral health status based upon new analyses of NIDR survey data and within the context of biological, behavioral and societal factors which may be important to a more comprehensive understanding of this subject. This presentation has been expanded for publication in a peer reviewed journal along with the other symposium papers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00587-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Quality of Life

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Redford, Maryann	Public Health Specialist	EODPP, NIDR
Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.17	.15	.02

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oral diseases and conditions are highly prevalent and the progressive consequences of these are not only physical, but economic, social, and psychological. The relation of oral health to overall quality of life has gained increasing recognition as an important area of scientific investigation. Branch staff have participated on NIH committees to broaden the depth and scope of quality of life research.

This research program represents a series of projects undertaken conceptualize oral quality of life as well as to describe and then quantify the functional, psychological, and social consequences of oral disorders and their treatment. Extensive literature reviews and consultations with external experts have been conducted in an effort to improve the measurement and interpretation of oral quality of life. Staff have worked with NCHS to improve ways of determining disability in relation to the oral cavity. Based upon a review and synthesis of literature published by staff in 1992, oral quality of life is being reassessed for the specific audience of aging veterans in a position paper for the Department of Veteran's Affairs.

Qualitative analyses have begun on focus group discussions conducted on a sample of women who perceived that oral problems had impacted their life in long-term ways. Analysis of the focus group transcriptions indicate that oral problems can alter an individual's self-image, activities, and life choices. Results will be useful for the development of quantitative measures in future investigations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00588-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bone Loss in the Oral Cavity and its Relation to Skeletal Bone Health

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Redford, Maryann Public Health Specialist EODPP, NIDR

COOPERATING UNITS *(if any)*

National Institute of Arthritis and Musculoskeletal and Skin Diseases
Bowman Gray School of Medicine

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.40	.25	.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bone loss in the oral cavity is a significant problem in the United States. In the dentate, oral bone loss may manifest as a loss of tooth support. In edentate individuals, osteopenia may augment local anatomic, biological, and mechanical factors resulting in extensive ridge atrophy. There have been speculations in the medical and dental literature that generalized skeletal osteopenia may be conducive to accelerated loss of oral bone. Thus, skeletal osteopenia may influence the need for and outcome of periodontal, pre-prosthetic, and implant surgical procedures. Conversely, there is evidence that oral assessment may provide a measure of skeletal bone health and, thus, have predictive, diagnostic, or therapeutic value for osteoporosis.

This research program represents a series of projects undertaken as follow-up to an NIDR and NIAMS sponsored workshop on osteoporosis and oral bone loss in August, 1992. A special supplement to the Journal of Bone and Mineral Research spotlighting the workshop is being finalized which will ensure the accurate and timely science transfer of the presentations and research recommendations to the extramural community.

Another key activity in this area of research focuses on analyses of data derived from collaboration with scientists at Bowman Gray School of Medicine. Now that the computation of fractal-based indices of trabecular bone patterns from existing dental radiographs is complete, analyses will be conducted to assess if these measures correlate with race, age, menopausal status, and medically diagnosed osteoporosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00589-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Orofacial Trauma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Oldakowski, Richard	Computer Programmer	EODPP, NIDR
Bhat, Mohandas		EP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.16	.16	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The consequences of orofacial trauma can be among the most permanent of oral conditions and diseases. Trauma (as a consequence of injuries to the face and mouth resulting from falls, sporting activities or abuse) and prevention of it have recently received more attention in research. Based on earlier pilot studies in the Branch, staff collaborated with the American Dental Association on the Survey of Dental Practice to obtain an estimate of the number of patients seen in the private dental practice for orofacial trauma. Analyses of these data were completed, presented at the 1993 IADR and have been accepted for publication in a professional journal. A portion of the 1991 NHIS contained questions about participation of children and youth in sports and their use of head and mouth protection. These data have been analyzed and are being prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00590-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluating Oral Health Status As It Relates to Problematic Eating Behaviors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Redford, Maryann Public Health Specialist EODPP, NIDR

COOPERATING UNITS *(if any)*

University of California, San Francisco

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The nutritional status of the elderly has been studied extensively but little is known about how oral health status relates to problematic eating behaviors among nursing home residents. Because of a dearth of information, dentistry is often excluded from inter-disciplinary strategies for treating dysphagia. If oral health problems are identified as contributing to malnutrition, weight loss, or the eventual placement of a feeding tube, then appropriate oral health interventions may improve the quality of life for nursing home residents.

Literature reviews have been conducted to identify research questions and hypotheses. Collaborations have been established with extramural scientists as preparation for investigations in this area. An initial project in collaboration with an extramural scientist is beginning which will examine the role of oral health status in the development of problematic eating disorders among nursing home residents. Existing oral health data will be linked to relevant clinical, social, cultural, and environmental data which are part of a parent study being conducted at the University of California, San Francisco. Interpretation of the results of this study should augment the rationale for improved recognition and treatment of presenting dental needs within this underserved population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00591-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Treatment Plans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Redford, Maryann	Public Health Specialist	EODPP, NIDR
Bronk, Matthew	Summer IRTA	EODPP, NIDR

COOPERATING UNITS *(if any)*

University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.37	.32	.05

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Substantial variation has been documented in dentists' assessments of caries, in dentists' decisions to intervene, and in the selection of treatment recommended and ultimately received by the patient. The factors associated with this variation are not well documented.

The purpose of this project is to obtain a description of the psychosocial factors involved in dentists' decisions to recommend certain treatment plans, and the patient's willingness to accept these treatment proposals. The initial phase of this study centers on qualitative analysis of focus group discussions conducted utilizing a subsample of dentists and patients participating in a study of dentists' restorative treatment decisions at the University of North Carolina, Chapel Hill. Analysis of the focus group transcriptions will be useful in refining hypotheses and data collection instruments for a quantitative phase to follow.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00592-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Research on Oral Health Education and Health Promotion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Paik, Da-il	Staff Fellow	EODPP, NIDR
Maeda, Naoko	Staff Fellow	EODPP, NIDR
Small, John S.	Public Health Advisor	EODPP, NIDR
Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Nowjack-Raymer, Ruth	Public Health Research Specialist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	1.75	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oral health education is an integral part of health promotion. Research on ways to improve content, channels of distribution, appropriateness as well as the need to identify specific audiences and to identify their needs is a primary focus of the Branch. Science transfer activities to help ensure that research based information is used in preparation of education materials and other communications also is integral to Branch activities. Adapting research findings to the needs of the audience, be they legislative, professional, general public or specific high risk populations is an important outcome of research conducted by NIDR. The Branch takes the NIDR lead in evaluating scientific literature for translation and interpretation for the varied audiences. The outcomes of these scientific evaluations result in published literature reviews in texts and journals, lectures and consultation. Major areas of emphasis are community water fluoridation, multiple modalities of fluoride, dental sealants as well as prevention of other oral diseases and conditions, and strategies for effective health education and promotion.

Staff have been working with outside investigators to develop a survey instrument to evaluate knowledge, opinions and practices for use in determining the basis upon which education and preventive regimens could be established at the local and state levels. Staff also have been working with an outside investigator to evaluate the content of health education text books to determine the extent, nature and appropriateness of oral health information being taught and how this information supports oral heatlh objectives in Healthy People 2000. The Branch is working with three international guest scientists in the areas of health education and health promotion. One is analyzing a survey from Korea on knowledge, opinions, and practices to establish the basis for a health education-promotion program, another is adapting infection control materials for use in Japan.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00593-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

International Health Promotion, Disease Prevention, and Epidemiologic Research

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Nowjack-Raymer, Ruth E.	Public Health Research Specialist	EODPP,NIDR
Horowitz, Alice M.	Education Specialist	EODPP
Gift, Helen C.	Chief, DPHP Branch	EODPP
Kleinman, Dushanka V.	Deputy Director	NIDR
Paik, Da-il	Guest Researcher	EODPP
Maeda, Naoko	Guest Researcher	EODPP

COOPERATING UNITS *(if any)*

World Health Organization

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.646	1.346	.30

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Numerous international initiatives which focus on improved disease prevention/health promotion research and enhanced science transfer internationally exist and include collaboration with investigators of the WHO International Collaborative Study II; authorship of articles for international publication; participation in international meetings as presenters and invited speakers, and as technical consultants; and the editing of a book on oral health promotion with an international focus. Additional, numerous guest researchers have been hosted by and have collaborated with staff in the development of research protocols for the initiation of studies. Scientific interchange has also been facilitated through the coordination of seminars, lecturers, meetings for international guests and visitors with a broad range of agencies, organizations and universities. Health education materials developed by NIDR have been made available to international organizations and agencies to enhance rapid science transfer.

The subject matter of the international initiatives cover diverse public health topics which include preventive dentistry, dietary fluoride supplements for preschool age children, community water fluoridation, primary prevention of oral disease, infection control, and curriculum development focused on oral disease prevention.

Additionally, the development of materials designed to mobilize oral health professionals, both domestically and internationally, to respond to the HIV pandemic continues. A training manual includes a chapter on the conduct of epidemiologic studies and surveillance activities of oral manifestations of HIV infection and a section on strategic planning for the prevention and control of HIV infection. Another manual, designed for use by chief dental officers, highlights the principles of developing country-specific approaches to responding to the disease. The World Health Organization (WHO) has announced that the theme for World Health Day 1994 is oral health. Events, year long, will focus on the importance of oral health as part of total health. Activities which foster science transfer and research toward the prevention of oral diseases are being planned in collaboration with the WHO and international organizations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00594-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Baby Bottle Tooth Decay

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Oldakowski, Richard	Computer Specialist	EODPP, NIDR
Kaste, Linda M.	Senior Staff Fellow	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.15	.10	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Baby bottle tooth decay is a specific concern in oral health promotion. It is more often a concern for children from minority and low income families. One staff member is collaborating with an outside investigator on a pilot, community-based study regarding the prevention of baby bottle tooth decay in a Hispanic population. The purpose of this project is to develop measurement approaches to assess knowledge and behaviors of parents. Ultimately, these assessments will be used to develop interventions to decrease baby bottle tooth decay and to determine if the interest of an Hispanic community can be focused on preventing baby-bottle tooth decay.

Analyses of the 1991 NHIS and of the 1993 NHANES III data are being conducted to gain information on inappropriate baby bottle feeding practices. Results describe the use of fluids other than water for bedtime bottle by age and socioeconomic status for children 6 months through 5 years of age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00595-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Research Toward Preventing Periodontal Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Nowjack-Raymer, Ruth E.	Public Health Research Specialist	EODPP, NIDR
Gift, Helen	Chief, DPHP Branch	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR
Mercer, Paula	Computer Specialist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.646	1.346	.30

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Self care remains the most effective approach for the prevention of periodontal diseases. Two studies of the Disease Prevention and Health Promotion Branch address the prevention of periodontal diseases, 1. an analysis of National Health Interview Survey data to explore the knowledge of US adults regarding the signs and symptoms of gum disease to establish baseline information from which appropriate and targeted educational campaigns may be developed, and 2. a study of two different school-based approaches to prevent gingivitis in teenagers.

The 1990 Heath Promotion Supplement to the National Health Interview Survey included a section on oral health and was administered to 41,104 respondents ages 18 years and older. Data were analyzed to compare the respondents knowledge of signs and symptoms of gum disease by selected sociodemographic and dental variables which included education, race, ethnicity, dental visit history, dentate status. Findings have been presented and a report has been accepted for publication.

A two year study of teenagers was conducted in York County, Virginia to determine the effectiveness of a self-assessment of gingival bleeding approach to the prevention of gingivitis compared with a plaque control approach. Both the plaque control and self-assessment of bleeding groups received an interactive manual describing the procedures they were to perform, classroom-based instruction and individual instruction specific to their needs. The individual instruction was reinforced following a 12 month interim oral examination. Preliminary findings show that while the two approaches did not differ, there were improvements in the gingival health status of both groups.

Ongoing consultation and assistance is provided for the planning and development of research conferences, symposia and research groups that focus on research related to periodontal disease prevention and oral health status improvement. Presentations are made as a part of science transfer efforts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00596-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Orofacial Pain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robinson, Dina	Health Promotion Research Specialist	EODPP, NIDR
Lipton, James	Special Asst. to Dir. EODPP	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.72	.57	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data analysis and interpretation of the orofacial pain supplement questionnaire of the 1989 National Health Interview Survey have been performed. The first paper from this effort provided national estimates of the prevalence of reported orofacial pain symptoms for the U.S. adult, civilian, non-institutionalized population as well as the corresponding sociodemographic distribution for each prevalence rate. A second analysis is on behavioral responses to orofacial pain symptoms for individuals who had experienced pain in the jaw joint and in front of the ear, pain in the face and cheek, burning sensations in the mouth and tongue or a combination of these symptoms for at least two months out of the past six months. Information has been assessed regarding (1) worries about oral and general health; (2) perceived health status; (3) specific health care sought; (4) coping behaviors; and (5) effects of pain on daily activities. The age and gender distribution have been determined by symptom group and behavioral response category.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00597-01 DPHP

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Research on Healthy People 2000

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gift, Helen C.	Chief, DPHP Branch	EODPP, NIDR
Horowitz, Alice M.	Education Specialist	EODPP, NIDR
Oldakowski, Richard	Computer Programmer	EODPP, NIDR
Small, John S.	Public Health Advisor	EODPP, NIDR

COOPERATING UNITS *(if any)*

Centers for Disease Control
Chief Dental Office

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.47	.37	.10

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oral health is a primary focus of Healthy People 2000, the nation's objectives for the 1990's. NIH is the co-lead agency for the oral health objectives and in that role assumes a responsibility for research to 1) monitor progress and 2) establish relevant programs to achieve the objectives. Staff of DPHPB serve on working groups, prepare reports and represent NIH on all Healthy People 2000 activities. All reports to the Assistant Secretary require analyses of existing data and reviews of literature to assure scientifically based responsiveness. Key topic areas this year have been use of fluorides, dental sealants, dental visits, nursing homes and orofacial trauma. In an effort to extend science transfer to the broader community, staff have developed papers related to objectives in Healthy People 2000 for publication in professional journals, and have worked with NCHS in the development of measures for upcoming survey which will improve assessment of the objectives.